**DOCTOR OF PHILOSOPHY**

# Cognitive and synaptic dysfunction in a mouse model of Huntington's disease

Mitchell, Ruth C.

*Award date:*
2012

DOCTOR OF PHILOSOPHY

# Cognitive and synaptic dysfunction in a mouse model of Huntington's disease

Ruth C. Mitchell

2012

University of Dundee

# Cognitive and synaptic dysfunction in a mouse model of Huntington's Disease

by

**Ruth C. Mitchell**

**A thesis submitted to the University of Dundee for the degree of Doctor of Philosophy**

# Table of Contents

**Table of Contents**……..i

**List of Tables**……...……viii

**List of Figures**……..…viii

**Abbreviations**……..xi

**Acknowledgements**……...……....xiii

**Declarations**……..…….....xiv

**Abstract**……...…...xv

**CHAPTER 1: HUNTINGTON'S DISEASE**……...…..……...1

**1.1:** Introduction……1

**1.1.1:** The physiological role of huntingtin protein……...2

**1.1.2:** The pathology of Huntington's disease……...4

**1.1.3:** Symptoms of Huntington's disease……7

**1.2:** Rodent models of Huntington's disease……...10

**1.2.1:** Transgenic models of Huntington's disease……11

**1.2.1.1:** The R6/2 model……..11

**1.2.1.2:** The R6/1 model……..13

**1.2.1.3:** YAC models……...……13

**1.2.1.4:** BAC models……...……14

**1.2.2:** 'Knock-in' models……...….16

**1.2.2.1:** 80 CAG mouse model……16

**1.2.2.2:** $Hdh^{Q92}$ and $Hdh^{Q111}$ mouse model…….……17

**1.2.2.3:** $Hdh^{Q140}$ mouse model……18

**1.2.2.4:** $Hdh^{Q150}$ mouse model……18

**1.2.3:** Summary……...……19

**CHAPTER 2: The hippocampus and synaptic plasticity**…….....21

**2.1:** Introduction……...……....21

**2.1.1:** Structure and anatomy of the hippocampus……..21

**2.2:** Synaptic plasticity in the hippocampus……22

**2.2.1:** LTP : A historical perspective……...24

**2.2.2:** Paired-pulse facilitation……...…….28

**2.2.3:** The induction of LTP……...……..……...28

**2.3:** LTP and memory…….....29

**CHAPTER 3: Episodic memory**…….34

**3.1:** Introduction……..34

**3.1.1:** What is memory?.....34

**3.1.2:** The hippocampus and episodic memory……...36

**3.2:** Studies of memory dysfunction in animals……..38

**3.3:** Do animals have episodic memory?.....40

**3.4:** Using the intrinsic novelty seeking behaviour of rodents to investigate memory...45

**3.4.1:** Standard novel object recognition task……...……..45

**3.4.2:** The ‘what’, ‘where’ and ‘which’ of episodic memory……...…..48

**3.4.3:** Extending novelty detection to look at memory for place and context….50

**CHAPTER 4.: GABA and Huntington’s disease**…….....…….….....59

**4.1:** Introduction……..59

**4.2:** GABA and the $GABA_A$receptor……...……...59

**4.2.1:** Functional domains of $GABA_A$ receptors……...……..60

**4.2.2:** Physiological role of $GABA_A$ receptors……63

**4.3:** $\alpha_5$- $GABA_A$ receptors influence cognition……...64

**4.3.1:** Synaptic versus extrasynaptic receptors……68

**4.4:** $GABA_A$ receptor and Huntington's disease……..……..……69

**4.4.1:** $GABA_A$ receptor trafficking and the regulation of synaptic strength…...72

**4.4.2:** HAP1 expression and function……..……72

**4.4.3:** HAP1 mediates delivery of $GABA_A$ receptors to the plasma membrane.73

**CHAPTER 5: Experiment 1: Characterisation of the motor phenotype of $Hdh^{Q111}$ mouse model of Huntington's disease**……..78

**5.1:** Introduction……..78

**5.2**: Methods……79

**5.2.1:** Breeding and genotyping of the $Hdh^{Q111}$ mouse…….....79

**5.2.2:** Rotarod…….…….79

**5.2.3:** Circular runways…….....82

**5.2.4:** Activity box…….....82

**5.3:** Results: Rotarod…….……..85

**5.3.1:** $Hdh^{Q111}$ mice show no progressive weight loss…….……..…85

**5.3.2:** $Hdh^{Q111}$ mice show a mild motor phenotype on the rotarod (2 and 3 months)……85

**5.4:** Results : Circular runways…….……...……...89

**5.4.1:** $Hdh^{Q111}$ mice show no locomotor deficits at 13 months…….……..89

**5.5:** Results : Activity box……...91

**5.5.1:** Mobile counts……91

**5.5.2:** Exploratory rearing……..……93

**5.5.3:** Static counts……..……....……...93

**5.5.4:** Active time…….……...96

**5.5.5:** Mobile time……...96

**5.6:** Discussion…...……99

**CHAPTER 6: Experiment 2 : Characterisation of the cognitive phenotype of the Hdh$^{Q111}$ mouse**…….....……...102

**6.1:** Introduction : Huntington's disease and memory……..……102

**6.1.1:** Cognitive deficits in human patients of Huntington's disease……102

**6.1.2:** Cognitive deficits in animal models of Huntington's disease…….....103

**6.2:** Methods…….……108

**6.2.1:** Assessment of episodic memory: long protocol…...……..108

**6.2.1.1:** Subjects……...……108

**6.2.1.2:** Apparatus…….……...……108

**6.2.1.3:** Behavioural testing……...109

**6.2.1.4:** Habituation……...110

**6.2.1.5:** Object recognition task……...…….111

**6.2.1.6:** Object-place task……..111

**6.2.1.7:** Object-context task……...112

**6.2.1.8:** Object-place-context task…….113

**6.2.1.9:** Data collection and analysis…….113

**6.2.2:** Assessment of episodic memory: short protocol……...….116

**6.2.3:** 24 hour novel object recognition task…….……116

**6.2.3.1:** Subjects……...…….117

**6.2.3.2:** Apparatus…….……...…….117

**6.2.3.3:** Habituation……...117

**6.2.3.4:** 24 hour novel object recognition task……..118

**6.2.3.5:** Data collection…….....118

**6.3:** Results: Assessment of episodic memory (2 months)…….....120

**6.3.1:** Object recognition task……...……120

**6.3.2:** Object-place and object-context…….……..……..121

**6.3.3:** Object-place-context task: Episodic memory……...……..122

**6.4:** Results: Assessment of episodic memory (13 months)……...……...…126

**6.4.1:** Object recognition task …….....126

**6.4.2:** Object-place and object-context……...……...127

**6.4.3:** Object-place-context task: Episodic memory…….....131

**6.5:** Results : Assessment of episodic memory, short protocol…….132

**6.5.1:** Object recognition task……...……....132

**6.5.2:** Object-place and object-context……..133

**6.5.3:** Object-place-context task: Episodic memory……...……..134

**6.6:** Results: 24 hour novel object recognition task ……...…….……....137

**6.6.1:** 24 hour novel object recognition task (2 month)……...……137

**6.6.2:** 24 hour novel object recognition task (1 month).…….…….……138

**6.7:** Discussion……...……...141

**CHAPTER 7: Experiment 3: Characterisation of the electrophysiological phenotype of the Hdh$^{Q111}$ mouse**……...….147

**7.1:** Huntington's disease and deficits in LTP…….……...……..147

**7.2:** Methods….…….……149

**7.2.1:** Hippocampal brain slice preparation…….....149

**7.2.2:** Solution application……...…….149

**7.2.3:** Generation of fEPSPs……..150

**7.2.4:** fEPSP analysis……...…….151

**7.2.5:** Input – output function……151

**7.2.6:** Paired pulse facilitation……...152

**7.2.7:** LTP induction……..152

**7.2.8:** Measurement of LTP……...155

**7.2.9:** Statistics and analysis……..155

**7.3:** Results…..……...156

**7.3.1:** Input/ output function……..156

**7.3.2:** Paired pulse facilitation……...156

**7.3.3:** LTP is impaired in the $Hdh^{Q111}$ mouse model of Huntington's disease..158

**7.4:** Discussion……...……...160

**CHAPTER 8: Experiment 4: Cognitive and LTP deficit is rescued by an $\alpha_5$-$GABA_A$ receptor inverse agonist**……...163

**8.1:** Introduction……163

**8.1.1:** Inverse agonists of the $\alpha_5$-$GABA_A$ receptor enhance cognition and LTP……..164

**8.1.2:** Specific targeting of the $\alpha_5$ subunit in an animal model of cognitive decline……...167

**8.2:** Methods ……168

**8.2.1:** Drug application – Cognition tests……..168

**8.2.2:** Drug application – LTP…….……169

**8.3:** Results…….……...169

**8.3.1:** Episodic memory (long protocol) $\alpha_5$IA drug treatment…….....169

**8.3.2:** Episodic memory (short protocol) $\alpha_5$IA drug treatment…….....173

**8.3.3:** 24 hour novel object recognition task : $\alpha_5$IA drug treatment…..……175

**8.3.4:** The effects of the $\alpha_5$-$GABA_A$ receptor inverse agonist $\alpha_5$IA on hippocampal LTP of WT and $Hdh^{Q111}$ mice…….178

**8.3.4.1:** The influence of the stimulus protocol on the magnitude of LTP……178

**8.3.4.2:** The magnitude of LTP following a sub-threshold stimulus is enhanced by an $\alpha_5$-$GABA_A$ receptor "inverse agonist……178

**8.3.4.3:** The impairment of LTP in 2 month old $Hdh^{Q111}$ mice is rescued by an $\alpha_5$-$GABA_A$ receptor inverse agonist……...183

**8.4:** Discussion…….……...……..185

**CHAPTER 9: Summary and Conclusions**…….……..……190

**REFERENCES**……193

# List of Tables

**1.1:** Transgenic models of Huntington's disease…...……..……..15

**1.2:** Knock-in models of Huntington's disease……..…….20

# List of Figures

**2.1:** A sagittal section of the rodent hippocampus……..23

**2.2:** Mechanisms of LTP…….27

**3.1:** Schematic diagrams representing the novel object, object place, location memory, object context and object-place-context tasks…….....56 - 58

**4.1:** Structure and heterogeneity of $GABA_A$ receptors……...62

**5.1:** Representation of genotyping results……..80

**5.2:** Photograph of a rotarod apparatus……...81

**5.3:** Photograph of the circular runway apparatus……..83

**5.4:** Photograph of the activity box apparatus……84

**5.5:** $Hdh^{Q111}$ mice demonstrate a mild motor phenotype on the rotarod…….88

**5.6:** $Hdh^{Q111}$ mice show no locomotor deficits in the circular runways (13 months).90

**5.7:** $Hdh^{Q111}$ mice show similar mobile counts in the activity box (13 months)……92

**5.8:** $Hdh^{Q111}$ mice show similar exploratory rearing in the activity box (13 months)……..94

**5.9:** $Hdh^{Q111+/+}$ mice show increased freezing in the activity box (13 months)……..95

**5.10:** $Hdh^{Q111}$ mice show similar active time counts in the activity box (13 months)..97

**5.11:** $Hdh^{Q111}$ mice show similar mobile time counts in the activity box (13 months)……..98

**6.1:** Representation of the apparatus used to assess episodic memory…….115

**6.2:** Representation of the apparatus used in the 24 hour novel object recognition task…….119

**6.3:** $Hdh^{Q111}$ mice show impairments in episodic memory at 2 months……...124

**6.4:** The exploration behaviours of $Hdh^{Q111}$ mice are unaffected at 2 months……125

**6.5:** The cognitive deficits of $Hdh^{Q111}$ mice are progressive……129

**6.6:** $Hdh^{Q111}$mice do not show any major differences in exploration at 13 months.130

**6.7:** The shortened protocol can be used to assess impairments in episodic memory in $Hdh^{Q111}$ mice at 2 months……..135

**6.8:** The exploration behaviours of $Hdh^{Q111}$ mice are unaffected at 2 months…….136

**6.9:** $Hdh^{Q111}$ mice show impairments in long-term memory at 2 months…….139

**6.10:** $Hdh^{Q111+/-}$ mice show no cognitive deficits at the age of 1 month…….140

**7.1:** A field extracellular postsynaptic potential (fEPSP)…….154

**7.2:** Input/out function and PPF is normal in the $Hdh^{Q111}$ mice (2 months)…….157

**7.3:** LTP is impaired in both homozygous and heterozygous 2 month old $Hdh^{Q111}$ mice ……..159

**8.1:** $\alpha_5$IA greatly improves the cognitive deficit of $Hdh^{Q111+/-}$ mice in the episodic memory task……..172

**8.2:** $\alpha_5$IA greatly improves the cognitive deficit of $Hdh^{Q111+/-}$ mice in the shortened version of the episodic memory task…….174

**8.3:** $\alpha_5$IA greatly improves the cognitive deficit of $Hdh^{Q111+/-}$ mice in the 24 hour novel object recognition task…….177

**8.4:** A comparison of LTP induced by a 4-TBS and 3-TBS protocol……..180

**8.5:** In WT hippocampal brain slices sub-threshold LTP is facilitated by $\alpha_5$IA…...181

**8.6:** The $\alpha_5$-$GABA_AR$ inverse α5IA has no effect on LTP induced by 4-TBS in wild type hippocampal slices…….182

**8.7:** $\alpha_5$IA rescues the deficit of LTP evident in $Hdh^{Q111+/-}$ hippocampal slices……..184

# ABBREVIATIONS

| | |
|---|---|
| **AChBP** | Acetylcholine binding protein |
| **AP5** | Aminophosphonovaleric acid |
| **BAC** | Bacterial artificial chromosome |
| **BDNF** | Brain derived neurotrophic factor |
| **CA** | *Cornu Ammonis* |
| **$Ca^{2+}$** | Calcium |
| **CAG** | Glutamine |
| **CANTAB** | Cambridge Neuropsychological Test Automated Battery |
| **CVLT** | California verbal learning test |
| **DG** | Dentate gyrus |
| **DNMTS** | Delayed non-match to sample |
| **EC** | Entorhinal cortex |
| **EPSP** | Excitatory postsynaptic potential |
| **fEPSP** | field excitatory postsynaptic potential |
| **GABA** | γ-aminobutyric acid |
| **HAP1** | Huntingtin-associated protein |
| **HD** | Huntington's disease |
| **$Hdh^{Q111+/+}$** | Homozygous $Hdh^{Q111}$ |
| **$Hdh^{Q111+/-}$** | Heterozygous $Hdh^{Q111}$ |
| **HFS** | High frequency stimulation |
| **$5HT_3$** | 5-hydroxytryptamine |
| **IA task** | Inhibitory avoidance task |
| **i.p** | intraperitoneal injection |
| **IPSC** | Inhibitory postsynaptic currents |

| | |
|---|---|
| **KIF5** | Kinesin family of motor proteins 5 |
| **LTD** | Long term depression |
| **LTP** | Long term potentiation |
| **$Mg^{2+}$** | Magnesium |
| **ms** | milliseconds |
| **MSN** | Medium spiny neurons |
| **MWM** | Morris Water Maze |
| **$Na^{+}$** | Sodium |
| **nACh** | nicotinic acetylcholine receptor |
| **NII** | Neuronal intranuclear inclusions |
| **NMDA** | N-Methyl-D-aspartic acid |
| **PPD** | Paired pulse depression |
| **PPF** | Paired pulse facilitation |
| **PTP** | Post tetanic potentiation |
| **WAIS-R** | Wechsler Adult Intelligence Scale-Revised |
| **WMS** | Wechsler memory scale |
| **WT** | Wild type |
| **YAC** | Yeast activated chromosome |

# Acknowledgements

Firstly, I would like to thank my supervisors Susann Schweiger, Rosamund Langston, Jeremy Lambert and Delia Belelli for their guidance and advice throughout the course of my PhD studies. Thanks in particular go to Ros for the endless hours she spent injecting mice during the drug trials. Without this invaluable help I'm sure I would still be timing the exploration patterns of my mice now!

Thanks to all members of the Division of Neuroscience past and present who have helped throughout the course of my PhD. Thanks to Lori-An Etherington, for the crash course in electrophysiology in the first 3 weeks of my PhD before she went off on her latest mission… medical student, postdoc, super mum – I can't keep up! An extra thanks goes to Linda Gallacher, for assistance in clipping and the hours of singing/ dancing/ general banter in the 'Girl's Lab.' It was a huge help when Linda answered my panicked emails/ phone calls requesting various bits of data during the final run up to the thesis hand in, much appreciated!!

I would have not made it to the end if it were not for my fantastic family. Special thanks goes to my parents who have provided online computer support, meals on wheels, not to mention a shoulder when it just got too much! I am eternally grateful.

Finally, I would like to thank my husband, Andy, who has humoured my perpetual student habit with ease - I promise to get a real job soon! His superbly timed deliveries of 'super scrumptious layers of chocolate and caramel mousse, toffee sauce and squidgy brownie, topped with chocolate coated shortcake biscuit' desserts made writing up a much more enjoyable experience!

Now for some Gin…

# Declarations

I hereby declare that the following thesis is based on the results of investigations conducted by myself and that this thesis is of my own composition. Work other than my own is stated with reference to the researcher or their publications. This body of work has not previously been presented for a higher degree.

Ruth C. Mitchell

I certify that Ruth C. Mitchell has completed nine terms in experimental research in the Division of Neuroscience, Medical Research Institute, University of Dundee. She has fulfilled the conditions of the Ordinance General No. 39 of the University of Dundee and is qualified to submit this thesis in application for the degree of Doctor of Philosophy.

Prof. Susann Schweiger

Dr. Rosamund Langston

Prof. Jeremy Lambert

Dr. Delia Belelli.

# Abstract

Huntington's disease (HD) is a neurodegenerative disorder characterised by an unstable polyglutamine repeat expansion within the *Huntingtin* gene. Although clinical diagnosis of HD relies on the manifestation of a motor phenotype, cognitive symptoms often appear prior to diagnosis. This study has characterised the motor, cognitive and electrophysiological phenotypes of the homozygous and heterozygous $Hdh^{Q111}$ mouse models of HD. Although the heterozygous $Hdh^{Q111}$ mouse is more clinically relevant to the human disorder, it has received little attention in previous studies.

Assessments of the motor phenotype of the $Hdh^{Q111}$ mouse were inconclusive. However, $Hdh^{Q111}$ mice exhibited a mild motor phenotype on the rotarod, showing hyperactivity at 2 and 3 months. Subtle changes within the hippocampus are thought to underlie the cognitive abnormalities that characterise the early stages of HD. A series of recognition tasks were used to assess the episodic memory of the $Hdh^{Q111}$ mouse. Although these tasks had been used to assess the ability of rats to discriminate the 'what', 'where' and 'when' of episodic memory, this was the first time that they had been successfully utilised in mice. $Hdh^{Q111}$ mice showed impairments in episodic memory as early as 2 months. In mouse models, cognitive deficits are often accompanied by impairments in hippocampal synaptic plasticity, the molecular correlate of learning and memory. In agreement, $Hdh^{Q111}$ mice showed impairments in long-term potentiation (LTP) at 2 months.

A novel, shortened version of the protocol was developed to accurately assess the changes in cognition in the small development window available. A hippocampus-dependent 24-hour novel object recognition task was also used to assess the integrity of

the hippocampus. Hippocampal function in 1 month old $Hdh^{Q111}$ mice was not significantly different from that in wild type mice. The early cognitive deficits present in the $Hdh^{Q111}$ mice were progressive, with cognitive deficits spreading to include the individual components of episodic memory by 13 months. Drugs inhibiting the function of $\alpha_5$-$GABA_A$ receptors are known to enhance cognition and hippocampal LTP. In agreement, the LTP and cognitive deficits of the $Hdh^{Q111}$ mouse were rescued following treatment with the $\alpha_5$-$GABA_A$ receptors selective inverse agonist $\alpha_5$IA.

Importantly, the clinically relevant heterozygous $Hdh^{Q111}$ mice exhibited an identical phenotype to homozygous $Hdh^{Q111}$ mice indicating that, reminiscent of the human disorder, only one copy of the mutant gene is necessary to produce abnormalities associated with the disorder, further supporting the validity of the $Hdh^{Q111}$ mouse as a clinically relevant model of HD. Collectively, this thesis provides evidence that $\alpha_5$-$GABA_A$ receptors antagonists have the potential to improve cognitive function in HD.

# 1: Huntington's disease

## 1.1: Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that affects muscle coordination (chorea), and leads to cognitive decline and psychiatric problems (for more details on the symptoms of HD, see section 1.1.3). The disorder is characterised by an unstable glutamine (CAG) trinucleotide repeat expansion which occurs within the open reading frame of exon 1 of the *Huntingtin* gene (Cummings *et al.*, 2006) (.NB. The following nomenclature is used throughout this thesis: *huntingtin* - mouse gene; huntingtin - mouse protein; *Huntingtin* - human gene; Huntingtin - human protein). Generally, unaffected individuals have less than 36 CAG repeats, however greater than 36 repeats results in the generation of HD. The age of onset and severity of the disease are determined by the length of the CAG repeat expansion (Duyao *et al*., 1993). Although adult-onset HD most often occurs in middle age, juvenile onset HD results either from a CAG repeat length of more than 55 or in individuals who are homozygous for the *Huntingtin* mutation, and in these cases symptoms can appear before the age of 20 (Nance and Myers, 2001). In addition, if the gene is passed down the paternal germline genetic anticipation can occur, thereby increasing the number of CAG repeats and decreasing the age of onset as the gene is passed through the generations (Duyao *et al*., 1993). In Scotland 1: 10,000 people suffer from HD, although this figure varies between regions. Currently, there are approximately 850 patients of HD in Scotland, with 4000 – 6000 people living with the risk of inheriting the disorder (Scottish Huntington's Association).

## 1.1.1: The physiological role of huntingtin protein

The physiological role of the Huntingtin protein is unclear, however various experimental approaches have been used to investigate normal Huntingtin function and its' possible involvement in the pathogenesis of HD. In order to further elucidate the physiological role of Huntingtin during development, mice with a targeted deletion of the *huntingtin* gene were generated (Nasir *et al*., 1995; Duyao *et al*., 1995; Zietlin *et al*., 1995). Homozygous knock-out of the *huntingtin* gene results in embryonic death, however mice heterozygous for the mutation survive until adulthood. Heterozygous knock-out mice showed significant neuronal loss in the subthalamic nuclei. In a set of behavioural tests it was found that heterozygous knock-out mice exhibited increased spontaneous motor activity when compared to control animals, echoing the chorea exhibited in human patients of the disorder (Nasir *et al*., 1995). The heterozygous mice showed impaired spatial learning in the Morris Water Maze (MWM) test (for more details on MWM, see section 6.1.2).

Following on from these studies, a study by White *et al.* (1997) generated knock-in mice in which the polyglutamine tract of the murine *huntingin* gene was extended by introducing an expanded human CAG repeat to create mice that expressed either reduced levels of huntingtin by the introduction of a neomycin cassette ($Hdh^{neoQ50}$), or wild type ($Hdh^{Q50}$) levels of huntingtin with 50 CAG repeats (for more details on knock-in mice, see section 1.2.2 below). Mice homozygous for $Hdh^{neoQ50}$ were either stillborn, or died shortly after birth (White *et al*., 1997). In addition, the subsequent examination of the homozygous pups revealed abnormal brain development. By contrast, brain development was normal in heterozygous and homozygous $Hdh^{Q50}$ mice

suggesting that huntingtin, in the endogenous or mutated form is critical to the formation of the central nervous system (White *et al*., 1997).

Again instead of completely deleting the *huntingtin* gene, a further study assessed the consequences of reducing huntingtin expression (Auerbach *et al*., 2001). Knock-in mice were generated in which the CAG length was reduced (CAG20) and/ or expanded (CAG111): $Hdh^{neoQ20}/Hdh^{neoQ20}$, $Hdh^{neoQ20}/Hdh^{null}$ and $Hdh^{neoQ20}/Hdh^{neoQ111}$. Through careful breeding $Hdh^{neoQ20}/Hdh^{null}$ and $Hdh^{neoQ20}/Hdh^{neoQ111}$ mice were able to survive until adulthood, but exhibited developmental defects as demonstrated by reduced body weight and enlarged cerebral ventricular volume (Auerbach *et al*., 2001). Movement abnormalities were evident in the $Hdh^{neoQ20}/Hdh^{neoQ111}$ mice from 2 months of age, including hind-limb clasping, progressing to limb stiffness, difficulty "walking", seizures and eventual paralysis as the mice aged. None of these abnormalities were present in control animals, or in any of the animals that expressed normal levels of huntingtin in at least one allele, demonstrating the critical role of huntingtin expression in embryonic development (Auerbach *et al*., 2001).

Further studies have examined the localization of Huntingtin in the brain (DiFiglia *et al*., 1995; Gutekunst *et al*., 1995; Sharp *et al*., 1995; reviewed in Cattaneo *et al*., 2005). Results indicated that Huntingtin is expressed widely throughout the brain and peripheral tissue (DiFiglia *et al*., 1995; Sharp *et al*., 1995), with higher expression in the cortex and cerebellum than in the striatum (Gutekunst *et al*., 1995) The widespread distribution perhaps suggests that Huntingtin plays a general 'housekeeping' role in a variety of cells (DiFiglia *et al*., 1995; Sharp *et al*., 1995). Wild type Huntingtin is expressed in cell bodies, dendrites, axons and nerve terminals, but not in nuclei (Sharp

*et al*., 1995), with highest expression in the pyramidal cells of the cortex (Gutekunst *et al*., 1995). In addition, analysis of the subcellular localization of Huntingtin revealed that the protein is strongly associated with microtubules, perhaps indicating a role in the anchorage, or transport of intracellular proteins (Gutekunst *et al*., 1995). Furthermore, a small amount of staining at dendritic spines indicated that Huntingtin may perhaps play a role in neurotransmission (Gutekunst *et al*., 1995). DiFiglia *et al.* (1995) also demonstrated using sucrose density gradients that Huntingtin can be detected in fractions which are enriched with vesicle-associated proteins, including the vesicle membrane protein synaptophysin, perhaps indicating a role for Huntingtin in vesicular transport.

Collectively these studies suggest that the normal function of the *Huntingtin* gene is required for embryonic development, neurogenesis and neurological development (Nasir *et al*., 1995; White *et al*., 1997; Auerbach *et al*., 2001). In addition, the widespread distribution throughout the brain, the periphery and its' subcellular localisation indicates that Huntingtin may play a role in a variety of different cell types (DiFiglia *et al*., 1995; Sharp *et al*., 1995). Such roles may include the anchorage, or transport of intracellular proteins (Gutekunst *et al*., 1995), synaptic transmission (Gutekunst *et al*., 1995), or vesicular transport (DiFiglia *et al*., 1995).

## 1.1.2: The pathology of HD

HD is caused by the expansion of a CAG repeat in the *Huntingtin* gene. The embryonic lethal phenotype of the huntingtin knock-out mice (Duyao *et al*., 1995; Nasir *et al*., 1995; see section 1.1.1) suggests a loss of function mechanism is unlikely. Therefore, in order to determine the mechanism by which the expanded CAG repeat causes HD, a

study by Davies *et al* (1997) assessed the neuropathology of the R6 transgenic mouse model of HD (for more details on the R6 mouse models, see section 1.2.1.1 and 1.2.1.2). Immunohistochemical studies in control animals indicated that, in agreement with previous studies (DiFiglia *et al*., 1995; Sharp *et al*., 1995), Huntingtin is expressed in multiple regions throughout the brain including the cerebral cortex, striatum and cerebellum and expression is limited to the cytoplasm and membrane (Davies *et al*., 1997). However, in contrast, immunohistochemical studies in the transgenic animals revealed that mutated Huntingtin formed dense inclusions within neuronal nuclei (Davies *et al*., 1997). These neuronal intranuclear inclusions (NIIs) were present in large numbers within neurons of the cerebral cortex, striatum, cerebellum, with fewer inclusions expressed in other areas, including the hippocampus, of transgenic mouse models (Davies *et al*., 1997). Furthermore these studies showed that in both transgenic mice and post mortem human patients with HD, antibodies detecting the C-terminus of Huntingtin showed staining only in the cytoplasm while antibodies detecting N-terminal Huntingtin demonstrated staining within the nucleus (Davies *et al*., 1997; DiFiglia *et al*., 1997). These studies suggest that the N-terminal fragment of mutant Huntingtin is cleaved and translocates to the nucleus to form NIIs. This proposal is supported by biochemical studies showing that N-terminal fragments of mutant Huntingtin form insoluble aggregates in the cortex and striatum (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997). Interestingly, the translocation of Huntingtin protein to the nucleus occurs just prior to the onset of the overt symptoms of HD, implicating a role for the NIIs in the generation of neurological dysfunction (for symptoms of HD, see section 1.1.3) (Davies *et al*., 1997). Furthermore, NIIs are present in symptomatic, but absent in pre-symptomatic patients of HD (DiFiglia *et al*., 1997). Collectively these studies suggest that the formation of aggregates within nuclei of transgenic

Huntington's models and human Huntington's before the appearance of motor symptoms is an important step in the development of HD (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997).

Although the pathogenesis of HD appears to involve the cytoplasmic cleavage, release and nuclear localisation of Huntingtin, it is not definitive that the protein aggregation is toxic and it is possible that the NIIs may instead act as a defence mechanism against huntingtin-induced cell death rather than being the cause of the disease (Saudou *et al.*, 1998). Saudou *et al.* (1998) have developed an *in vitro* model in order to assess the role of huntingtin expression in neuronal survival. Wild type or mutant huntingtin was transfected into striatal neurons and the survival rates of these were quantified. The study indicated that striatal neurons transfected with mutant huntingtin showed clear signs of neurodegeneration, the rate of which increased proportionally with the length of the CAG repeat (Saudou *et al*., 1998). In contrast, transfection of mutant huntingtin into hippocampal neurons maintained in cell culture did not result in an increased rate of neurodegeneration (Saudou *et al*., 1998). In addition, it was found that blocking components of the apoptotic pathway prevented neurodegeneration, indicating an apoptotic mechanism is involved in huntingtin-induced neurodegeneration (Saudou *et al*., 1998). In agreement with previous studies (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997), Saudou *et al.* (1998) also demonstrated that fragments of mutant huntingtin are transported from the cytoplasm into the nucleus. However, in contrast to the previous studies which suggested that nuclear huntingtin accumulation leads to neurodegeneration through the formation of NIIs (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997), Saudou *et al.* (1998) suggested that the formation of NIIs is instead a parallel process, unrelated to the mechanism that eventually results

in neurodegeneration. In agreement, results indicated that although NIIs developed in both striatal and hippocampal neurons *in vitro*, mutant huntingtin-induced apoptosis was only evident in striatal neurons, which shows that the presence of inclusions is not always sufficient to induce apoptosis (Saudou *et al*., 1998). Furthermore, when the formation of NII is suppressed, huntingtin-induced death is accelerated (Saudou *et al*., 1998).

Collectively, these studies suggest that mutant Huntingtin acts within the nucleus to induce neurodegeneration (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997; Saudou *et al*., 1998). However it is unknown whether the NIIs formed by mutant Huntingtin lead to neurodegeneration (Davies *et al*., 1997; DiFiglia *et al*., 1997; Scherzinger *et al*., 1997) or instead act as a protective mechanism against the toxic mutant Huntingtin protein (Saudou *et al*., 1998).

### 1.1.3: Symptoms of HD

In humans, symptoms of HD are progressive and usually appear in the third to fifth decades of life. HD produces 3 types of symptoms: (i) motor disturbances such as chorea, dystonia and clumsiness (ii) psychiatric features including mood swings and depression and (iii) cognitive impairment, characterised by attention difficulties and short- and long-term memory impairments which later on develop into dementia (Montoya *et al*., 2006). There is currently no treatment to halt the progression of HD, however medication is available that is able to manage the symptoms of the disorder (Killoran and Biglan, 2012).

The first motoric sign of HD is dystonia, which is characterised by slow movements with an increased muscle tone, leading to abnormal posture. These movement disturbances initially start as twitches of the fingers, toes and facial muscles but gradually spread to other muscles as the disease progresses. Involuntary choreatic movements develop in the latter stages of the disorder, and involve rapid and irregular movements of the limbs, trunk and face (Sturrock and Levitt, 2010). Patients also develop hypokinesia (decreased body movement) and akinesia (inability to initiate movement), which gradually leads to a slower pace of life (Roos, 2010). Impairments in voluntary movement also occur in HD with both hyperkinesia and hypokinesia, leading to difficulties in walking and standing, resulting in frequent falls (Sturrock and Levitt, 2010). In addition, impaired voluntary control and worsening involuntary movement of the mouth, tongue and lips lead to problems with speech and swallowing, causing weight loss that may eventually lead to malnutrition (Sturrock and Levitt, 2010). For individuals with juvenile onset HD, the symptoms are similar but the age of onset is reduced. It is thought that the deficits in motor function are mediated predominantly by the prominent degeneration of neurons within in the striatum (Melone *et al.*, 2005).

Psychiatric features are also present in the early stages of the disease, often before any motor symptoms become evident, and are the most variable aspect of the clinical phenotype of HD. The most frequent warning sign of the disorder is depression. This is often complicated by additional environmental stressors, including fear of the future and alterations of family or relationship dynamics (Sturrock and Levitt, 2010). In the later stages of the disease psychosis may appear, often combined with cognitive decline (Roos, 2010). Other psychiatric symptoms include aggression and compulsive behaviour, the latter of which can exacerbate pre-existing addictive behaviours such as

gambling or alcoholism (Roos, 2010). For many sufferers and their families, these psychiatric symptoms are among the most distressing aspects of the disease, often affecting daily functioning and constituting reason for institutionalisation (Roos, 2010).

Cognitive decline is another of the main indicators of HD and subtle changes in attention, semantic verbal fluency, working memory and episodic memory can be evident prior to the emergence of the more overt motor phenotype in patients (Hahn-Barma *et al.*, 1998; Lawrence *et al.*, 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007; see section 6.1.1) and mouse models of HD (Lione *et al.*, 1999; Van Raamsdonk *et al.*, 2005; Brooks *et al*., 2006; Pang *et al*., 2006; Nithianantharajah *et al*., 2008; Simmons *et al*., 2009; see section 6.1.2).

In the early stages of the disease patients demonstrate problems with concentration, working memory, episodic memory, attention and emotional processing (Montoya *et al*., 2006). Cognitive deficits become more severe as the disorder evolves. One of the earliest deficiencies in HD is the speed of thought processing and motor skills, with tasks that were previously menial becoming more tiring, with more effort required to achieve the same outcome (Paulsen, 2011). The cognitive deficits gradually deteriorate throughout the course of the disease, eventually resulting in dementia (Montoya *et al*., 2006). Although the clinical manifestation of HD relies on the appearance of motor abnormalities, it has been suggested that the cognitive changes place the greatest burden on families that are affected by HD (Hamilton *et al*., 2003; Williams *et al*., 2010). In addition, the cognitive and behavioural symptoms of Huntington's patients generally appear at least 15 years prior to diagnosis based on motor deficits (Paulsen *et al*., 2008). Subtle changes within the hippocampus are thought to underlie the cognitive

abnormalities that characterise the early stages of HD (Rosas *et al.*, 2003). Furthermore, cognitive deficits in mouse models of HD are often accompanied by impairments in hippocampal synaptic plasticity, the molecular correlate of learning and memory (see section 2.3) (Hodgson *et al.*, 1999; Usdin *et al.*, 1999; Murphy *et al.*, 2000; Milnerwood *et al.*, 2006; Lynch *et al.*, 2007). In order to identify possible drug targets, it would be beneficial to fully characterise the early cognitive changes that are associated with prodromal HD. As a result, it is the cognitive deficits (section 6.1) and abnormalities in synaptic plasticity (section 7.1) in HD that I will concentrate on and will be discussed in more detail in the relevant sections of this thesis.

## 1.2: Rodent models of HD

Since the mutation in the *Huntingtin* gene was first discovered in 1993 (MacDonald *et al.*, 1993), HD research has been aided considerably by the generation of a variety of rodent genetic models, allowing the exploration of early pathological, molecular and cellular abnormalities associated with the disorder. Such approaches may lead to the identification of potential therapeutic targets and the preclinical evaluation of prospective therapies in the treatment of HD and other polyglutamine repeat diseases. The following section will summarise the most widely used mouse models of HD. The available rodent models are transgenic models (ectopic expression of the *Huntingtin* mutation; Table 1.1) and knock-in models (mutated *Huntingtin* is knocked into the endogenous murine *huntingtin* gene; Table 1.2). Transgenic models include the R6 lines, which were the first genetic models of HD to be generated and still remain one of the most widely used (Mangiarini *et al.*, 1996; Davies *et al.*, 1997; Carter *et al.*, 1999; Lione *et al.*, 1999; Murphy *et al.*, 2000; Lüesse *et al.*, 2001; Sun *et al.*, 2002; Naver *et al.*, 2003; Hickey *et al.*, 2005; Morton *et al.*, 2000; Morton *et al.*, 2005; Stack *et al.*,

2005; Milnerwood *et al*., 2006; Pang *et al*., 2006; Nithianantharajah *et al*., 2008). In addition, there are 2 full length transgenic models of HD; one of the most studied is the yeast activated chromosome (YAC) expressing mutant (YAC72 and YAC128) human Huntingtin (Hodgson *et al*., 1999; Slow *et al*., 2003; Van Raamsdonk *et al*., 2005a/b) while the other uses a bacterial activated chromosome (BAC) and expresses mutant Huntingtin with 97 CAG repeats (Grey *et al*., 2008). There are classical knock-in models which differ mainly according to CAG repeat length (from 48 – 200) within the endogenous *huntingtin* gene of the mouse and include the $Hdh^{Q80}$ (Shelbourne *et al*., 1999; Usdin *et al*., 1999), $Hdh^{Q92}$ (Wheeler *et al*., 2000), $Hdh^{Q111}$ (Wheeler *et al*., 2000; Wheeler *et al*., 2002; Lynch *et al*., 2007), $Hdh^{Q140}$ (Menalled *et al*., 2003; Simmons *et al*., 2009) and the $Hdh^{Q150}$ (Lin *et al*., 2001; Yu *et al*., 2003; Brooks *et al*., 2006; Heng *et al*., 2007) mouse models of HD.

## 1.2.1: Transgenic models of HD

### 1.2.1.1: The R6/2 model

The R6 mouse was the first successful transgenic model of HD and was created by Bates and colleagues by incorporating an exon 1 fragment of *Huntingtin* with a range of 141 – 157 CAG repeats, expressed from different locations in the mouse genome. (Mangiarini *et al*., 1996; Table 1.1). The lines R6/1 and R6/0 contain 1 exon fragment integrated into the murine genome. The R6/2 line contains 3 inserted fragments, but due to deletions in the flanking regions, the inserted transgene functions as a single insertion. Finally the R6/5 line contains 4 fragment inserts (Mangiarini *et al*., 1996). The following section will discuss the R6/2 and R6/1 lines.

The R6/2 mouse expresses an aggressive phenotype and has been characterised extensively; behavioural abnormalities are evident by 5 weeks, neuronal cell loss in the striatum by 12 weeks, followed by death by 12 - 15 weeks (Mangiarini *et al*., 1999; Hickey *et al*., 2005; Morton *et al*., 2005; Stack *et al*., 2005). Studies have shown that NIIs are present from postnatal day 1, forming initially in the cortex and in the *Cornu Ammonis* 1 (CA1) region of the hippocampus by 3 weeks, spreading to other hippocampal regions and the striatum by 8 weeks of age (Davies *et al*., 1997; Morton *et al*., 2000; Murphy *et al*., 2000; Sun *et al*., 2002; Stack *et al*., 2005). By the end stage of the disease, the R6/2 mouse displays widespread NIIs and aggregates (Morton *et al*., 2000; Stack *et al*., 2005). The appearance of the NIIs in the striatum correlates with the generation of motor abnormalities (Carter *et al*., 1999; Stack *et al*., 2005). In R6/2 mice motor impairments develop between 8 – 15 weeks including deficits in swimming, fore- and hind-limb coordination, balance, grip strength and sensorimotor gating (Carter *et al*., 1999; Lüesse *et* al., 2001; Stack *et al*., 2005). The R6/2 mice show a progressive decline in performance of the rotarod task as early as 6 weeks and are unable to maintain their balance on the beam for longer than 10 seconds by the age of 12 weeks (Lüesse *et al*., 2001; Hickey *et al*., 2005; Stack *et al*., 2005). In addition, R6/2 mice (8 weeks) are smaller in weight when compared to wild type mice (Hickey *et al*., 2005; Stack *et al*., 2005) and exhibit stereotypical behaviours such as hind limb clasping (6 weeks) (Mangiarini *et al*., 1996; Stack *et al*., 2005). However, prior to the emergence of the more overt motor impairments, cognitive testing has demonstrated that the R6/2 mouse shows progressive cognitive decline in the MWM and the T-Maze as early as 3.5 weeks (Lione *et al*., 1999; Lüesse *et al*., 2001; Morton *et al*., 2005; see section 6.1.2). Furthermore, R6/2 mice show impairments in hippocampal synaptic plasticity, the

molecular correlate of learning and memory, from 5 weeks (Murphy *et al*., 2000; see section 7.1).

### 1.2.1.2: The R6/1 model

Another model in the R6 line is the R6/1 mouse model of HD. As the R6/1 mouse contains only 116 CAG repeats (Mangiarini *et al*., 1996), the phenotype of this mouse is relatively mild when compared to the R6/2 mouse (Table 1.1). In common with the R6/2 mouse, NIIs form within the CA1 region of the hippocampus in 1 month old R6/1 mice, spreading to the CA3 by 3 months and the dentate gyrus by 7 months (Milnerwood *et al*., 2006). The R6/1 mouse exhibits weight loss and hind-limb clasping by the age of 19 – 23 weeks (Milnerwood *et al*., 2006) and decreased anxiety in the plus maze (24 weeks) (Naver *et al*., 2003). The R6/1 mouse also demonstrates deficits in spatial learning (12 weeks) and novel object recognition (12 – 14 weeks) (Pang *et al*., 2006; Nithianantharajah *et al*., 2008; see section 6.1).

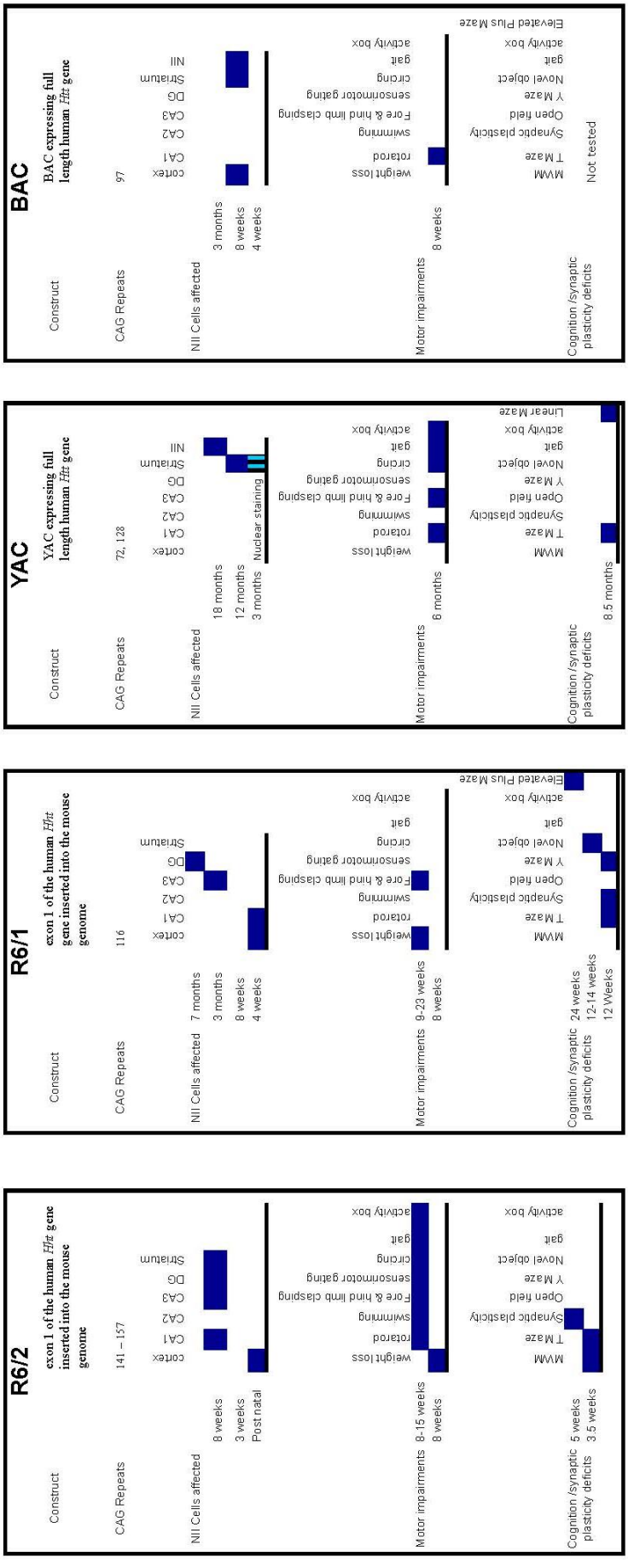
### 1.2.1.3 YAC models

A further method of creating mouse models of HD is to express the entire human *Huntingtin* gene including the CAG repeats in the 5'end of the gene under the control of the human promoter in a yeast artificial chromosome (YAC) vector system. YAC mouse strains expressing mutant (YAC72 and YAC128) *Huntingtin* were generated (Hodgson *et al*., 1999; Slow *et al*., 2003; Van Raamsdonk *et al*., 2005a; Table 1.1). Modest striatal neuronal loss is found at 12 months in the YAC128 model (Slow *et al*., 2003). Although NIIs are not evident until 18 months (Hodgson *et al*., 1999; Slow *et al*., 2003), both the YAC72 and YAC128 demonstrate increased nuclear Huntingtin staining within striatal neurons from 3 months (Van Raamsdonk *et al*., 2005a). Both models

show stereotypical behavioural abnormalities from 3 months, including circling, deficits in gait and hind-limb clasping (Hodgson *et al*., 1999; Slow *et al*., 2003). The YAC128 model of HD also shows deficits in performance on the accelerating rotarod and reduced locomotion in the activity box (6 months) (Slow *et al*., 2003; Van Raamsdonk *et al*., 2005b). In addition, cognitive tests have demonstrated that the YAC128 model demonstrates deficits in spatial learning (8.5 months; Van Raamsdonk *et al*., 2005b; see section 6.1).

### 1.2.1.4: BAC models

An alternative method of creating mouse models of HD is to express the full length human *Huntingtin* under the control of the endogenous *Huntingtin* regulatory machinery on the bacterial artificial chromosome (BAC) vector system (BACHD; Gray *et al*., 2008; Table 1.1). The BACHD mice generated had 97 CAG repeats and their brains demonstrated late onset atrophy (12 months) of the cortex and striatum (Gray *et al*., 2008). NIIs were found in the deep and upper cortical layers, with a few aggregates forming in the striatum at 12 months (Gray *et al*., 2008). BACHD mice demonstrated subtle impairments in the rotarod from 2 months (Gray *et al*., 2008). BACHD mice therefore provide a subtle model that demonstrates a slower progressive presentation of HD, without the normal aggregation of mutant Huntingtin.




**Mangiarini** *et al.*, 1996; **Davies** *et al.*, 1997; **Morton** *et al.*, 2000; **Murphy** *et al.*, 2000; **Sun** *et al.*, 2002; **Stack** *et al.*, 2005; **Carter** *et al.*, 1999; **Lüesse** *et al.*, 2001; **Hickey** *et al.*, 2005

**Mangiarini** *et al.*, 1996; **Milnerwood** *et al.*, 2006; **Naver** *et al.*, 2006; **Pang** *et al.*, 2006; **Nithianantharajah** *et al.*, 2008.

**Hodgson** *et al.*, 1999; **Slow** *et al.*, 2003; **Van Raamsdonk** *et al.*, 2005a; **Van Raamsdonk** *et al.*, 2005b.

**Gray** *et al.*, 2008.

**Table 1.1: Transgenic models of Huntington's disease. [Abbreviations :** *Htt* – **human** *Huntingtin* **gene; INN – intranuclear inclusions; CA1 -** *Cornu Ammonis* **1; CA3 –** *Cornu Ammonis* **3; DG – dentate gyrus; YAC – yeast artificial chromosome; BAC – bacterial artificial chromosome]**

## 1.2.2: ‘Knock-in’models

Due to the presence of both the mouse and human copies of the Huntingtin gene in the previously described models, it could be argued that transgenic models are not an accurate representation of the human disease. As a result, knock-in models of HD have been generated. Knock-in models are produced by replacing a portion of the mouse *huntingtin* gene with a mutant copy of the human gene, which contains a section with an expanded CAG repeat. Unlike the transgenic models, the knock-in mice only have 2 copies of the *huntingtin* gene – 1 wild type and 1 mutant – both of which are under the control of the endogenous mouse *huntingtin* promoter. Knock-in mice that contain 48 – 200 CAG repeats have been generated, a small selection of which will be discussed in the following section (Shelbourne *et al*., 1999; Usdin *et al*., 1999; Lin *et al*., 2001; Wheeler *et al*., 2000; Wheeler *et al*., 2002; Menalled *et al*., 2003; Yu *et al*., 2003; Brooks *et al*., 2006; Heng *et al*., 2007; Lynch *et al*., 2007; Simmons *et al*., 2009; Usdin *et al*., 2009) (Table 1.2). Generally, the phenotype of the knock-in mice is more subtle than the phenotype seen in transgenic models, however sensitive and careful testing can be utilised in order to demonstrate the early deficits associated with HD (Menalled *et al*., 2002; 2003).

### 1.2.2.1: 80 CAG mouse model

Mice expressing full-length mutant huntingtin protein were generated by replacing the endogenous mouse *huntingtin* gene with an expanded stretch of up to 80 CAG repeats, a length corresponding to that seen in juvenile-onset HD in humans (Shelbourne *et al*., 1999). These mice do not develop detectable nuclear inclusions or motor disturbances (Shelbourne *et al*., 1999), but show a striking hyper-aggressive behaviour reminiscent of the behavioural abnormalities of early stage human patients of HD. In addition, these

mice demonstrate impairments in hippocampal synaptic plasticity (8 – 14 months) (Usdin *et al*., 1999; see section 7.1).

### 1.2.2.2 : $Hdh^{Q92}$ and $Hdh^{Q111}$ mouse model

Mice were generated in which exon 1 of the mouse *huntingtin* gene was replaced by a chimeric mouse/ human exon 1 including either 92 ($Hdh^{Q92}$) or 111 ($Hdh^{Q111}$) CAG repeats (Wheeler *et al*., 2000; Wheeler *et al*., 2002; Table 1.2). The striatum of the $Hdh^{Q92}$ and $Hdh^{Q111}$ mice was subsequently examined in order to detect the presence of neurodegeneration. Although neither of these models displayed overt striatal neurodegeneration, abnormal striatal pathology is evident. In young mutant $Hdh^{Q92}$ and $Hdh^{Q111}$ mice (1.5 months) huntingtin protein is confined to the cytoplasm of striatal neurons. However, by 4.5 months, the huntingtin protein has translocated to the nucleus in both the $Hdh^{Q92}$ and $Hdh^{Q111}$ mouse lines (Wheeler *et al*., 2000). In $Hdh^{Q111}$ heterozygous mice, prominent nuclear huntingtin immunoreactivity is observed at 5 months of age. At 17 months both homozygous and heterozygous $Hdh^{Q111}$ mice display prominent NIIs, and aggregates are found in the globus pallidus and substantia nigra pars reticulata (Wheeler *et al*., 2002). Behavioural tests carried out to assess weight gain, clasping, exploratory behaviour, gait and rotarod deficits did not produce any significant differences between wild type and mutant mice up to the age of 17 months (Wheeler *et al*., 2000). However footprint analysis demonstrated that subtle motor deficits are apparent by 24 months in the $Hdh^{Q111}$ mouse (Wheeler *et al*., 2002). In addition, further studies have shown that $Hdh^{Q111}$ mice also display impairments in the long term potentiation of CA1 hippocampal neurons, from 2 months onwards (Lynch *et al*., 2007; see section 7.1).

### 1.2.2.3: $Hdh^{Q140}$ mouse model

A further knock-in model carrying 140 CAG repeats (Menalled *et al*., 2003; Table 1.2) displays weak nuclear staining and few aggregates within the striatum at 2 months. By 4 months nuclear staining is clearly more intense and aggregates are more widely distributed when compared to younger mice, with aggregates spreading to the striatum and hippocampus, increasing in density by 6 months (Menalled *et al*., 2003). The $Hdh^{Q140}$ mouse does not show any abnormal weight loss when compared to control. The $Hdh^{Q140}$ mouse shows increased locomotor activity and rearing at the age of 1 month, which is significantly reduced at the age of 4 months. Furthermore, these mice present with overt gait abnormalities by 12 months of age as demonstrated by a decrease in stride length (Menalled *et al*., 2003). In addition the $Hdh^{Q140}$ mouse model of HD also demonstrated cognitive deficits in the novel object recognition task (16 weeks; Simmons *et al*., 2009; see section 6.1).

### 1.2.2.4: $Hdh^{Q150}$ mouse model

The $Hdh^{Q150}$ knock-in mouse lacks foreign DNA sequences and has 150 CAG repeats inserted into exon 1 of the murine *huntingtin* homologue (Lin *et al*., 2001; Yu *et al*., 2003; Brooks *et al*., 2006; Heng *et al*., 2007; Table 1.2). In agreement with the human disorder in which the age of onset and severity of the symptoms are characterised by the number of CAG repeats, the $Hdh^{Q150}$ mouse model of HD displays a phenotype that is more severe than the aforementioned knock-in models. Nuclear huntingtin immunoreactivity is found at 27–29 weeks. At approximately 40 weeks of age, some striatal neurons express NIIs which gradually disperse so much so that, by 70 weeks of age, NIIs are expressed by most striatal neurons. The $Hdh^{Q150}$ mice display increased striatal axonal degeneration at 14 months when compared to wild type (Yu *et al*., 2003).

At 100 weeks, homozygous $Hdh^{Q150}$ mice exhibit weight loss, decreased motor activity, impaired performance on the accelerating rotarod, abnormal gait, clasping and abnormal performance on the balance beam (Heng *et al*., 2007). Tests to assess the cognitive performance demonstrated that $Hdh^{Q150}$ mice show impairments in performance in a test of attention at 26 weeks (Brooks *et al*., 2006).

## 1.2.3: Summary

As stated previously, as the mutant *huntingtin* is under the control of the mouse promoter and there are only two *huntingtin* copies in these animals, knock-in models most accurately represent the human disorder. The behavioural deficits are not as pronounced in the knock-in models when compared to other transgenic models and are difficult to test due to the lack of refined behavioural testing. However, with new and more sophisticated test settings the phenotype of the knock-in models can be studied more carefully to reveal early deficits. The slow progression of the disorder in these animals certainly provides an ideal model for the study of therapeutic interventions. For these reasons I have chosen the $Hdh^{Q111}$ mouse model of HD for this study and aim to characterise both motor and cognitive features in sophisticated test settings as well as to analyze potential electrophysiological deficits. In addition to the homozygous $Hdh^{Q111}$ mice, I will also assess the phenotype of the more clinically relevant heterozygous $Hdh^{Q111}$ mouse model of HD, which has received little attention in previous studies.

| $Hdh^{Q80}$ | | $Hdh^{Q92}$ | | $Hdh^{Q111}$ | | $Hdh^{Q140}$ | | $Hdh^{Q150}$ | |
|---|---|---|---|---|---|---|---|---|---|
| Construct | endogenous mouse *htt* gene replaced with extended CAG repeats | Construct | exon 1 of the mouse *hht* gene was replaced with mutant human exon 1 | Construct | exon 1 of the mouse *hht* gene was replaced with mutant human exon 1 | Construct | Insertion of CAG repeats in the mouse *htt* gene | Construct | Insertion of CAG repeats in the mouse *htt* gene |
| CAG Repeats | 80 | CAG Repeats | 92 | CAG Repeats | 111 | CAG Repeats | 140 | CAG Repeats | 150 |
| NII Cells affected | None | NII Cells affected | No overt striatal neurodegeneration, but htt translocates to the nucleus by 4.5 months. | NII Cells affected | No overt striatal neurodegeneration, but htt translocates to the nucleus and appears punctuate by 4.5 months. NIIs & aggregates (17 months) | NII Cells affected | Progressive build up of nuclear staining and aggregates (2 months); more widely distributed in older mice (6 months old) when compared to younger mice | NII Cells affected | nuclear aggregates (14 months) |
| Motor impairments | None | Motor impairments | No overt symptoms | Motor impairments | No overt symptoms up to 17 months. Abnormalities in gait (24 months). | Motor impairments | Decreased locomotor activity – increased activity (4 weeks) & decreased activity (12 weeks). Gait abnormalities (12 months) | Motor impairments | Deficits in rotarod performance, clasping, gait abnormalities (4 months) |
| Cognition /synaptic plasticity deficits | No cognitive features tested. Impairments in synaptic plasticity (8-14 months). | Cognition /synaptic plasticity deficits | No overt symptoms | Cognition /synaptic plasticity deficits | No reported cognitive symptoms up to 17 months. Abnormalities in synaptic plasticity (2 months) | Cognition /synaptic plasticity deficits | Impairments in novel object recognition (16 weeks) | Cognition /synaptic plasticity deficits | Deficits in 2 choice discrimination task (26 weeks) |
| Shelbourne *et al.*, 1999; Usdin *et al.*, 1999. | | Wheeler *et al.*, 2000; ; Wheeler *et al.*, 2002 | | Wheeler *et al.*, 2000; Wheeler *et al.*, 2002; Lynch *et al.*, 2007. | | Menalled *et al.*, 2003; Simmons *et al.*, 2009. | | Lin *et al.*, 2001; Yu *et al.*, 2003; Heng *et al.*, 2007; Brooks *et al.*, 2006. | |

Table 1.2 : Knock-in models of Huntington's disease. [Abbreviations : *htt* – mouse *huntingtin* gene; CAG - glutamine]

# 2: The hippocampus and synaptic plasticity

## 2.1: Introduction

Damage to the hippocampus is considered to contribute to the early cognitive deficits associated with HD (Rosas *et al.,* 2003). In addition, forms of synaptic plasticity such as long-term potentiation (LTP), considered to be a cellular correlate of memory (see section 2.3), have been widely studied in the hippocampus, and decreases in the magnitude of LTP in this brain region are thought to be associated with the cognitive deficits seen in mouse models of HD (Usdin *et al.*, 1999; Murphy *et al.*, 2000; Lynch *et al.,* 2007; Simmons *et al.,* 2009; see section 6.1). The following sections will describe the anatomy of the hippocampus and molecular mechanisms of synaptic plasticity.

### 2.1.1: Structure and anatomy of the hippocampus

The hippocampus is a brain region that is important in memory processes, including the retention of spatial information and memory for recent events. The role of the hippocampus in memory was brought to light in a landmark study by Scoville and Milner (1957). In this study, patient Henry Gustav Molaison (1926 – 2008), known worldwide only as H.M, underwent a bilateral removal of the medial temporal lobe, including the hippocampus, as a treatment for epilepsy. However, following the operation, H.M. developed severe anterograde and a partial retrograde amnesia (for more details see section 3.1.2). Interestingly, two additional case studies also underwent a bilateral medial temporal lobectomy. However, in these cases the hippocampus was spared. In contrast to H.M, short- and long-term semantic and episodic memory was spared when the hippocampus remained intact (Scoville and Milner, 1957). This

landmark study therefore demonstrated the importance of the hippocampal region in certain aspects of memory, in particular episodic memory (see section 3.1.2).

The primary input to the hippocampus is *via* the entorhinal cortex (EC) which projects to the dentate gyrus (DG) through the perforant pathway. The dentate gyrus projects to the CA3 *via* the mossy fibres, which in turn innervates the CA1 *via* the Schaffer collateral pathway known as the "trisynaptic" circuit (Anderson *et al*., 1971; Amaral and Witter, 1989; Amaral & Lavenex, 2007). This tri-synaptic pathway is primarily uni-directional (Figure 2.1). The CA1 neurons project to both the subiculum and to the deeper layers of the EC projecting widely to other brain regions including the temporal and frontal cortex, the amygdala and thalamus (Amaral *et al*., 1991; Amaral & Lavenex, 2007). Although the primary granule cells and pyramidal neurons are glutamatergic, the hippocampus also contains a network of GABA-ergic interneurons which modulate neuronal transmission (Amaral & Lavenex, 2007; Kasugai *et al*., 2010).

## 2.2: Synaptic plasticity in the hippocampus

Synaptic plasticity is the alteration of the efficiency of synaptic transmission, which may involve increases, or decreases in synaptic strength. Short-term forms of plasticity include paired-pulse facilitation (PPF), which occurs on the scale of hundreds of milliseconds, and post-tetanic potentiation (PTP) which may last several minutes (Bliss *et al*., 2007). Long-term forms of plasticity include long-term potentiation (LTP) and depression (LTD) that manifest as sustained increases and decreases in synaptic strength respectively and may last for several hours or longer (Bliss *et al*., 2007).

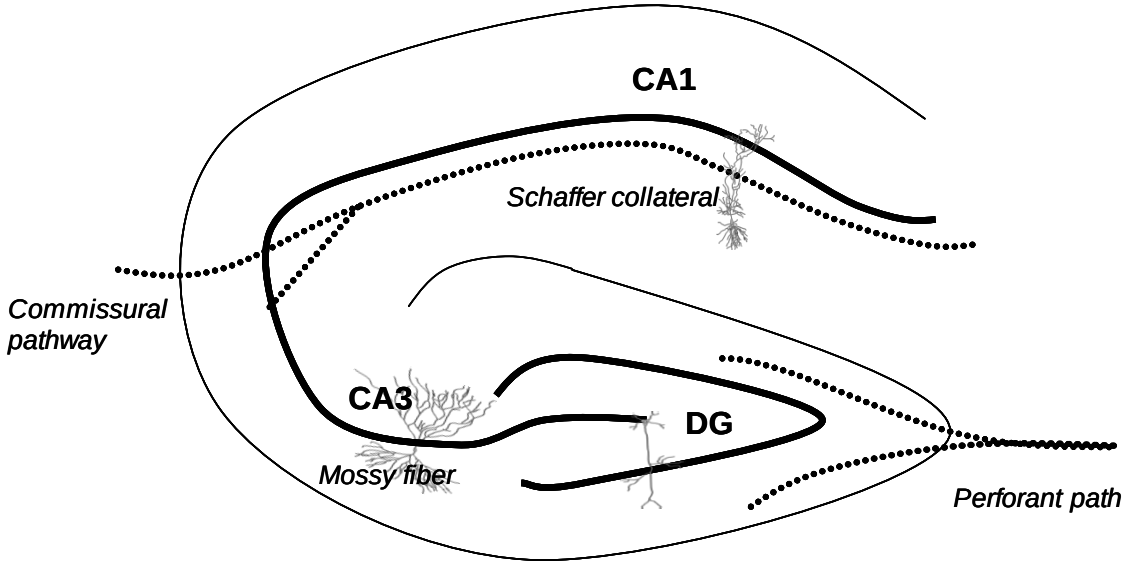



***Figure 2.1: A sagittal section of the rodent hippocampus.*** *Input emanates from the entorhinal cortex via the perforant path where it subsequently synapses with DG granule cells. The granule cells project to the CA3 region via the mossy fibres and then synapse with the pyramidal cells in this region. CA3 pyramidal cells then project to the CA1 pyramidal cells via the Schaffer collateral-commissural pathway.*

## 2.2.1: LTP: A historical perspective

At the end of the 19th Century, neurobiologists recognised that the number of neurons in the adult brain did not significantly increase with age, leading to speculation that memories did not result from the production of new neurons. In 1894, Santiago Ramon y Cajal suggested that learning results from strengthening the connections between existing neurons to improve the effectiveness of their communication (see Kandel, 2009). The Hebbian Theory introduced by Donald Hebb in 1949 (Cooke and Bliss, 2006; reviewed in Bi and Poo, 2001) echoed these ideas, speculating that cells may grow new connections, or undergo metabolic changes to enhance their ability for communication:

'…when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased' (for review, see Bi and Poo, 2001).

LTP was first discovered in the synapses between perforant path fibres and granule cells of the rabbit hippocampus (Bliss and Lømo, 1973). A single pulse of electrical stimulation to fibres of the perforant pathway caused the generation of excitatory postsynaptic potentials (EPSPs) in the cells of the DG. It was discovered that the postsynaptic cells' response to the single-pulse stimuli could be enhanced for a long period of time if a high frequency train of stimuli was delivered to the presynaptic fibres (Bliss and Lømo, 1973). The application of high-frequency stimulation (HFS) produced a long-lived enhancement of the response of the postsynaptic cell to subsequent single-pulse stimuli.

Since the discovery of LTP in the rabbit hippocampus, this form of synaptic strengthening has been observed in many pathways in the brain (reviewed in Cooke and Bliss, 2006). In the two most commonly studied pathways, the Schaffer collateral-commissural pathway and the perforant pathway, induction of LTP is dependent upon the activation of the NMDA receptors (Collingridge *et al*., 1983; Figure 2.2). Under normal basal conditions the NMDA receptor is subject to a voltage-dependent $Mg^{2+}$ block and so a low frequency stimulus to the Schaffer collateral pathway results in the generation of an AMPA receptor-mediated EPSP (Collingridge *et al*., 1983; Davies and Collingridge, 1989; reviewed in Cooke and Bliss, 2006). However, in the presence of the enhanced glutamate release that occurs at higher frequencies of stimulation, the influx of $Na^{+}$ through postsynaptic AMPA receptors results in a sustained depolarisation of the postsynaptic spine, consequently relieving the NMDA receptor $Mg^{2+}$ block, allowing $Ca^{2+}$ influx and the activation of $Ca^{2+}$-dependent enzymes (Cooke and Bliss, 2006). The importance of $Ca^{2+}$ in the induction of LTP was confirmed by Lynch and colleagues who demonstrated that injection of a $Ca^{2+}$ chelator into CA1 pyramidal cells blocks the induction of LTP (Lynch *et al*., 1983).

Inhibitory GABAergic interneurons which act on the postsynaptic spine usually act to limit the depolarisation by curtailing the EPSP, however the sustained depolarisation caused by the LTP induction protocol, activates inhibitory $GABA_B$ autoreceptors which subsequently leads to a reduction in GABA-mediated hyperpolarisation of the postsynaptic cells (Davies & Collingridge, 1996). In addition, $\alpha_5$-$GABA_A$ receptors (see section 4.2.1) are located extrasynaptically at the base of the spines, which receive excitatory glutamatergic input *via* NMDA receptors. Thus, $GABA_A$ receptors

containing the $\alpha_5$-subunit are strategically located to modulate the processing of the excitatory input (reviewed by Möhler, 2007).

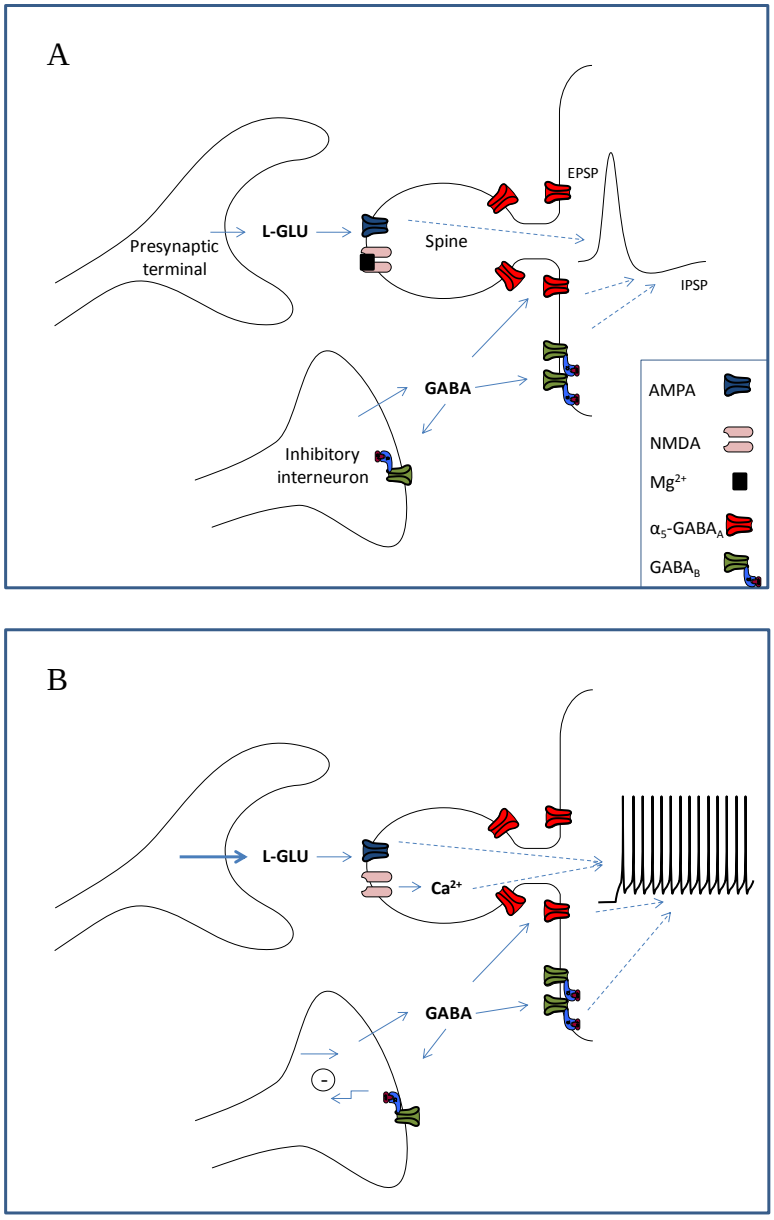



***Figure 2.2: Mechanisms of LTP.*** *A) Low frequency stimulation of the presynaptic cell releases glutamate (L-Glu) that then acts on AMPA receptors to evoke an EPSP. Low frequency stimulation also activates GABAergic interneurons, which release GABA that subsequently activates initially $GABA_A$ and then $GABA_B$ receptors, leading to the generation of an IPSP, which curtails the EPSP. $\alpha_5$-$GABA_A$ receptors are located extrasynaptically at the base of the spines, which receive excitatory glutamatergic input via NMDA receptors. At resting membrane potentials NMDA receptors are subject to $Mg^{2+}$ block and therefore provide little contribution to the synaptic response. B) High frequency stimulation results in the sustained depolarisation of the presynaptic cell, thus relieving the $Mg^{2+}$ block of the NMDA receptors, while the persistent release of glutamate during the tetanus enhances their probability of opening. GABA-mediated synaptic inhibition is reduced, thereby shifting the excitatory/ inhibitory balance. The NMDA receptor $Mg^{2+}$ block is further reduced, causing the NMDA receptor-mediated EPSPs to summate, generating LTP (Figure based on Bliss and Collingridge, 1993 and modified).*

## 2.2.2: Paired-pulse facilitation

Paired-pulse facilitation (PPF) occurs at CA1 hippocampal synapses when two presynaptic stimuli are given within 50 – 500 ms of each other and enhances the probability that neurotransmitter will be released (Debanne *et al*., 1996). The amplitude of the second synaptic response recorded is typically greater than that of the first stimulus. PPF is largely due to presynaptic mechanisms, in particular the transient increase in $Ca^{2+}$ generated by an incoming action potential (Schulz *et al*., 1994). The residual $Ca^{2+}$ remaining in the terminal after the first stimulus is combined with the $Ca^{2+}$ influx occurring from the second stimulus, thereby resulting in an enhancement in the magnitude of the second synaptic response. The magnitude of PPF is linearly related to the concentration of residual $Ca^{2+}$ within the terminal (Wu and Saggau, 1994).

## 2.2.3: The induction of LTP

In contrast to the *in vivo* preparations where the DG offers the most stable recording conditions, *in vitro* LTP has been most extensively studied in the Schaffer-commissural pathway. The hippocampal brain slice preparation (Skrede and Westgaard, 1971) has been a key experimental tool in advancing the understanding of both the pre- and post-synaptic mechanisms underlying LTP. The orderly nature of the neuronal cell bodies and zones of conductivity of the hippocampus allow electrophysiological recordings to be obtained from well-defined anatomical layers. Furthermore, stable intra- and extra-cellular recordings can be maintained for long periods of time thereby allowing rapid pharmacological manipulation of the extracellular environment. As a result, the CA1 of the hippocampus has become the prototypical site of mammalian LTP study (Cooke and Bliss, 2006).

LTP has traditionally been induced by delivering a tetanic stimulus (a train of 50-100 stimuli at 100 Hz or more) to the pathway of interest (Bliss and Collingridge, 1993). Additionally, LTP can also be induced by patterns of stimulations based on theta rhythms known to occur physiologically in the hippocampus of animals during learning (Bliss & Collingridge; 1993; Otto *et al*., 1991). LTP is expressed as a persistent increase in the size of the synaptic component of the neurally evoked response, recorded from individual cells, or a population of neurons. The increase in synaptic strength can be evaluated in terms of changes in the amplitude, or the slope of the extracellularly recorded field EPSP (fEPSP).

## 2.3: LTP and memory

Although LTP has primarily been studied in the hippocampus, it is also apparent in other regions of the brain such as cerebral cortex, cerebellum and amygdala in a variety of species (Rioult-Pedotti *et al.,* 2000, Coesmans *et al.,* 2004; reviewed in Cooke and Bliss, 2006). In the hippocampus, LTP has been proposed as a cellular and molecular correlate of learning and memory. A number of studies in rodents have correlated deficits in spatial memory with reductions in LTP both *in vitro* and *in vivo* (Morris *et al*., 1986; Tsien *et al*., 1996; Tang *et* al., 1999; Whitlock *et al*., 2006).

In 1986 Richard Morris and colleagues provided some of the first evidence that there was an association between spatial memory and hippocampal LTP. The spatial memory of rodents can be assessed in the MWM test (Morris *et al*, 1986). In this test rodents are trained to find a hidden platform in an opaque pool of water, then the platform is removed during a spatial transfer test. Normal rats exhibit a memory for the original position of the platform and therefore spend more time swimming in that location.

During a reversal test the platform is moved to another location in the pool and the ability of the rodent to learn the new position is assessed. Morris *et al.* (1986) demonstrated that rats that were chronically infused with the NMDA receptor antagonist aminophosphonovaleric acid (AP5) *via* a cannula inserted into the right ventricle prior to training, took longer to locate the platform, and took more indirect routes when compared to the control group. Furthermore, in contrast to control animals, rats in the AP5 group were able unable to learn the new position of the platform during the reversal test. Importantly, if rats were trained in the MWM prior to intra-ventricular infusion with AP5, they performed as well as controls, indicating an involvement of NMDA receptors in processing and storage of information (Morris *et al*., 1989). Moreover, AP5 inhibited the induction of *in vivo* hippocampal LTP, indicated a role of NMDA receptors in LTP induction (Morris *et al*., 1986).

As AP5 was delivered to the entire brain, it is possible that NMDA receptors in the neighbouring neocortex and other brain regions were also inhibited to a varying degree. However in support of a causative association between CA1 hippocampal LTP and spatial memory, Tsien *et al.* (1996) developed and utilised a mouse in which the gene that encodes the essential subunit for the NMDA receptor, the NMDAR1 subunit (Moriyoshi *et al.*, 1991), was exclusively deleted from the CA1 pyramidal cells in the mutant mouse (CA1-KO). Consistent with the knock out of the NMDA gene, whole-cell patch clamp techniques were utilised to demonstrate that the CA1-KO cell lacked the slow component of the excitatory postsynaptic current that is normally attributed to the NMDA receptor, whereas the early faster component, mediated through the AMPA receptor was intact (Tsien *et al*., 1996). Although basal synaptic transmission appeared normal in the CA1-KO mice, the application of tetanic stimulation failed to produce

LTP. In addition, it was evident that the CA1-KO mice were unable to demonstrate spatial learning in the MWM task (Tsien *et al*., 1996). Furthermore, when a transfer test was performed, in comparison to the control animals, CA1-KO mice did not demonstrate preference for the original position of the platform, further indicating deficits in spatial memory (Tsien *et al*., 1996).

In addition, non-spatial learning and memory has also been linked to LTP (Tang *et al.*, 1999). In this study, transgenic mice were generated in which the NMDA receptor 2B (NR2B) was overexpressed in the forebrain. Previously it has been shown that inhibition of the NMDA receptors prevents the induction of LTP (Bliss and Collingridge, 1993; Morris *et al*., 1986). Although NR2B-containing receptors have been shown to exist at both synaptic and extrasynaptic locations (Thomas *et al*., 2006), specifically, it has been demonstrated that only synaptic NMDA receptors are required for LTP, whilst LTD relies on both synaptic and extrasynaptic NMDA receptors (Papouin *et al*., 2012). In agreement with increased NR2B expression, Tang *et al.* (1999) demonstrated that the isolated NMDA-receptor-mediated field responses were significantly greater in the transgenic mice when compared to control, while the AMPA-mediated field EPSP responses remained unchanged. Furthermore, following tetanic stimulation, the magnitude of LTP was significantly greater in recordings made from brain slices derived from the transgenic mouse and this enhanced LTP could be blocked by the application of AP5 (Tang *et al*., 1999). Tang *et al.* (1999) subsequently subjected the NR2B transgenic mice to a series of non-spatial tests. The mice were initially tested in the novel-object-recognition task (for more detail, see section 3.4.1). Results showed that, at the retention interval of 1 hour, both control and transgenic animals showed similar preference for the novel object. However, when the retention

interval was increased to 1 or 3 days, the transgenic animals exhibited a higher preference for the novel object when compared to control, indicating that the transgenic mice have enhanced long-term memory (Tang *et al*., 1999). In addition, Tang and colleagues (1999) assessed the effect of increased NR2B expression on contextual (hippocampus-dependent) and cued fear (hippocampus-independent) conditioning. Results showed that transgenic animals demonstrated heightened contextual fear conditioning (Tang *et al*., 1999). In summary, this study demonstrates that over-expression of the NR2B subunit significantly enhances LTP and improves learning in the novel object recognition and fear conditioning tasks, indicating a role of LTP in non-spatial learning (Tang *et al*., 1999).

Although the aforementioned studies have provided a link between LTP and memory, it was not until 2006 that direct evidence demonstrated that hippocampal LTP is actually induced by learning. In a study by Whitlock *et al.* (2006), rats were tested in the hippocampal-dependent one-trial inhibitory avoidance (IA) task. Previous studies have shown that the IA task creates a memory trace and increased gene expression within the CA1 region of the hippocampus after only 1 trial (Impey *et al*., 1998; Taubenfield *et al*., 1999; Taubenfield *et al*., 2001). During the IA trial, the rats were placed within a light/ dark box, in which a foot shock was administered following entry into the dark side. Memory was quantified by measuring the subsequent avoidance of the dark chamber. Whitlock and colleagues (2006) demonstrated that, in common with LTP, acquisition of the avoidance response required the activation of NMDA receptors and the insertion of AMPA receptors into the synapse (Heynen *et al*., 2000). In agreement, Whitlock *et al.* (2006) also demonstrated that IA-trained animals showed increased protein levels of the GluR1 and GluR2 subunits of the AMPA receptor. Whitlock *et al.* (2006) used the

phosphorylation of serine residues in the GluR1 subunit of the AMPA receptor as a biomarker for LTP and demonstrated increased phosphorylation following IA training. Furthermore, this increased phosphorylation could be blocked by injection (i.p.) with an NMDA receptor antagonist (Whitlock *et al*., 2006). Whitlock *et al.* (2006) also examined fEPSPs in the CA1 region of the hippocampus and demonstrated that IA training produces an enhancement of fEPSP slope *in vivo*. In addition, in a small group of animals HFS was applied following IA training in an attempt to introduce saturating levels of LTP and it was determined that fEPSPs that showed enhancement following IA training showed less subsequent LTP in response to HFS. In summary, this study has demonstrated that IA training mimics the effects of HFS and causes an NMDA receptor dependent increase in AMPA receptor phosphorylation, delivery of AMPA receptors to the membrane and the subsequent increase in fEPSP slope. Furthermore the IA-induced increases in fEPSP slope occlude subsequent LTP by HFS *in vivo*. Collectively, these data suggests that IA-training induced LTP in the CA1 region of the hippocampus, providing further evidence for the link between LTP and memory (Whitlock *et al*., 2006).

# 3: Episodic memory

## 3.1: Introduction

Cognitive decline is one of the major symptoms of HD. Specifically, deficits in short/ long term recognition and episodic memory have been demonstrated in human patients (see section 6.1.1) and mouse models of HD (see section 6.1.2). Furthermore, these deficits have been linked to changes within the hippocampus (Rosas *et al.*, 2003). This section will therefore discuss the role of the hippocampus in memory, focusing in particular on recognition and episodic memory.

### 3.1.1: What is memory?

Memory is the storage and retrieval of information and experiences. Waugh and Norman (1965) segregated memory into primary (short term) and secondary (long term) memory. Primary memory was subsequently labelled as working memory (Baddeley and Hitch, 1974). During working memory, information is held transiently by the brain (short-term memory), long enough to make a decision, and is either consolidated by the brain for future retrieval (long-term memory), or lost. An example of working memory is memorising a phone number from a phone book immediately prior to dialing. Once dialing has been completed the numbers are forgotten. Long term memory includes both procedural (skill learning) and declarative (fact learning) aspects of memory. Procedural memory involves the unconscious storage of knowledge about the ability to perform a motor, or perceptual task, as in driving a car, or playing the piano. Once the skill has been learned over repeated practice sessions, these tasks can be completed implicitly, but are not necessarily easy to explain explicitly. On the other hand, declarative

memories are learned experiences that are accessible through a conscious awareness and encompass the ability to explicitly communicate facts and events to others.

Declarative memory is made up of semantic and episodic memory (Tulving, 1972). Semantic memory is based on facts: knowledge about the world is registered and stored until retrieval is required. An example of semantic memory could be 'Toyota manufactures cars'. This type of memory is explicit and can easily be communicated to others but does not necessarily contain any contextual information about the occasion where this fact was learned. Episodic memory requires an individual to remember a personal experience, *i.e.* 'I have a Toyota Yaris, I drove to St Andrew's last weekend and we had a picnic on the beach'. Episodic memory in humans requires an individual to be consciously aware of their self and their experiences. While a semantic memory is often formed by the repeated learning of facts, an episodic memory can be formed by a single exposure to a stimulus and therefore has not had the repeated exposures required in order to be established as a learned fact.

As mentioned above, human patients (see section 6.1.1) and mouse models of HD (see section 6.1.2) demonstrate deficits in short-long term recognition and episodic memory. In order to fully characterise these early cognitive changes, this thesis will experimentally explore declarative memory, in particular episodic memory, in the $Hdh^{Q111}$ mouse model of HD. Abnormalities within the hippocampus are thought to contribute to the early cognitive deficits in HD (Rosas *et al.*, 2003). The following section will therefore discuss the role of the hippocampus in episodic memory.

## 3.1.2: The hippocampus and episodic memory

As discussed previously, the role of the hippocampus in memory was determined when patient H.M developed amnesia following the removal of the medial temporal lobe, including the hippocampus (Scoville and Milner, 1957; see section 2.1). Further testing of H.M. revealed that he was unable to form new episodic memories for events (anterograde amnesia). He also was unable to demonstrate episodic memory for the 19 months prior to the surgery (retrograde amnesia) and had impairments in some episodic memories for 3 years prior to the operation. He was able to remember parts of his childhood, however the memories could be considered as semantic as although he was able to give generalised information about his parents he was unable to provide any specific memories of a given episode. This retrograde amnesia fits in with a popular theory that the medial temporal lobe, including the hippocampus, only plays a temporary role in memory. Memory for events that occurred a short time before the damage is impaired, but memory for events that occurred a long time before the damage, *i.e.* H.M.'s childhood, is spared (Alvarez and Squire, 1994). However as time has progressed the definitions of semantic and episodic memory have been refined and it is now doubted that H.M. ever had post-operative episodic memory (Corkin, 2002). It is possible that the memories he had from his childhood were in fact based on personal experiences that were repeated to him by family and friends and could therefore considered to be semantic memories of a personal event. Memories that have been relayed back by 'story-telling' are likely to be retrieved semantically so even the most convincing episodic memories of H.M. are not likely to require the hippocampus for processing. This suggests that although H.M. is unable to demonstrate episodic memory, his semantic memory for facts and events prior to his operation is intact.

Results from patients like H.M. who show retrograde amnesia following damage to the hippocampal complex provide support for the idea of memory consolidation, which proposes that what has been encoded is not instantly made permanent. This led to the multiple trace theory of memory (Nadel and Moscovitch, 1997) which suggests that certain types of memory, in this case episodic memory, are stored in the cortex but the hippocampus and related structures are required for the encoding and recovery of the memory. It was proposed that information is encoded in a distributed group of hippocampal neurons which then act as an index in order to direct retrieval to the correct memory traces in the cortex. In the case of H.M. where some of his 'episodic' memories are in fact semantic memories due to repeated exposure to the same event, re-activation of these memory traces would have resulted in the generation of newly encoded hippocampal memory traces, which are again sparsely distributed throughout the hippocampus. After the hippocampal damage that followed H.M.'s medial temporal lobe resection there is therefore more chance of any remaining hippocampal tissue to contain the index for that specific memory (Moscovitch *et al.*, 2005). This is in contrast to an early theory of the 'standard' model of memory consolidation (Marr, 1971) which states that information is registered in the neocortex and is bound into a memory trace in the hippocampus and related structures in the medial temporal lobes and diencephalon (Squire and Morgan, 1991; Squire, 1992). At first the hippocampus and related structures are required for the storage and recovery of memories but their contribution diminishes as time passes, until the neocortex alone is capable of sustaining the permanent memory trace and mediating its retrieval.

A further study by Vargha-Khadem *et al.* (1997) provided evidence for the role of the hippocampus in episodic but not semantic learning. The view of declarative memory as

a single unitary process suggests that any damage to the hippocampus would result in impairments in both semantic and episodic memory. However the study of patients who had sustained hippocampal damage early in life, before the knowledge base that characterises semantic memory had been established, demonstrated that this was not the case (Vargha-Khadem *et al*., 1997). All 3 patients involved in the study had normal intelligence and although they were able to demonstrate intact semantic memory as indicated by their ability to attain good general knowledge, all 3 patients were unable to retain information about events of their daily lives (*i.e.* impaired episodic memory). Deficits in spatial learning meant that they were unable to find their way around well experienced surroundings. Although 'well-experienced' surroundings imply the involvement of semantic memory, it is possible that the processing of spatial memory always requires the hippocampus, regardless of whether semantic or episodic memory is involved. One of the possible explanations of the apparent sparing of semantic memory but the absence of episodic memory in anterograde amnesia is that the basic memory functions of the underlying perirhinal and entorhinal cortices may be sufficient enough to process semantic information but the processing of context-rich episodic memories requires additional processing provided by the hippocampal circuit (Vargha-Khadem *et al.*, 1997).

## 3.2: Studies of memory dysfunction in animals

Studies in amnesic patients led to animal models of memory dysfunction. In an attempt to replicate the lesions in H.M.'s operation (Scoville and Milner, 1957), Mishkin (1978) performed a complete medial temporal lobe lesion in monkeys. These monkeys were subsequently tested using the hippocampus-dependent delayed non-match to sample (DNMTS) task. In this task the monkey is presented with a sample stimulus. After a

short delay, 2 test stimuli are presented, one of which is identical to that seen previously whereas the other is novel and the monkey is rewarded if it is able to correctly identify the novel object. Results showed that monkeys with combined lesions of the hippocampus and amygdala are significantly impaired in their ability to identify the novel object in this task when compared to control animals (Mishkin, 1978). In agreement with a lack of memory impairments in a patient with bilateral lesions of the amygdala in the study of Scoville and Milner (1957), monkeys with lesions of amygdala were unimpaired in the DNMTS task (Mishkin, 1978). In addition, although no such equivalent information existed in amnesic patients, monkeys with hippocampal lesions were also unimpaired in the DNMTS task (Mishkin, 1978). With the knowledge of episodic memory that is available today, the lack of impairment following hippocampal lesions could indicate that the DNMTS task is not an accurate model of episodic memory but as there was no available data at the time to contradict the findings of Mishkin (1978) it was concluded that damage to both the amygdaloid and hippocampal systems was necessary to produce memory deficits and, in the case of the memory impairments of H.M, both systems were connected (Mishkin, 1978).

However, later studies indicated that the technique used to produce the lesions in the original study by Mishkin (1978) had not only ablated the amygdaloid and hippocampal systems, but also the majority of the rhinal cortices too (Murray and Mishkin, 1986). Subsequently experiments were performed in which only the rhinal cortices were removed. Results indicated that it was in fact the perirhinal cortex that was critical for the successful completion of the DNMTS task. If lesions were restricted to the hippocampus and did not extend to the rhinal cortices, both monkeys (Meunier *et al*.,

1993) and rats (Otto and Eichenbaum, 1992) were capable of identifying the novel object, emphasising the role of the perirhinal cortex in the DNMTS task.

During the subsequent years, research focussed on the development of tests that would more accurately model episodic memory. Gaffan (1991) believed that the organisation of spatial stimuli was a critical determinant of human episodic memory. In humans, individual episodic memories can be discriminated between by reconstructing the scene in which that specific memory had occurred *i.e.* if an individual has lost their car keys they would picture the scene where they had last had the keys in order to locate them. As a result Gaffan's scene memory followed the DNMTS task as the next model of episodic memory (Gaffan, 1994). In this study monkeys were presented with complex visual scenes after which they received lesions of the fornix. Results indicated that, after surgery, the monkeys were unable to recognise the visual scenes they had experienced before their fornix lesion. In addition, the fornix-lesioned monkeys were impaired in their ability to learn new scenes after surgery when compared to control animals. Further studies in more controlled conditions suggested that it was in fact the associations of objects and their positions in their environment that was sensitive to lesions of the fornix (Gaffan and Harrison, 1989). This deficit is also present in rats with lesions of the hippocampus and will be discussed in more detail later (Ennaceur *et al*., 1997; Mumby *et al*., 2002; see section 3.4.3).

## 3.3: Do animals have episodic memory?

It has long been a question whether non-human species have the ability to demonstrate episodic memory. Everyone remembers where they were when the Twin Towers came down; your semantic memory would tell you that it happened on September 11$^{th}$ 2001

in New York City, whereas your episodic memory would bring back memories of coming out of your English class to find all the teachers crowded round the television in the Geography department to watch the news unfold. However it has long been debated as to whether or not animals possess the same kind of ability to mentally travel back and forward in time that humans take for granted. Developmental psychologists have argued that young infants do not have a conscious sense of self until the age of 4 and it has been suggested that although these children, like animals, are able to learn the sequence of ordered events, they are "unable to represent events as happening in unique temporal locations in their past" (McCormack & Hoerl, 1999). Mackintosh (1983) suggested that, although it was little understood, animals had the ability to "represent different attributes of their environments, to respond in terms of spatial, and even of abstract relationships between events, to store and rehearse information for later use." The guarded nature of this sentiment was echoed by Tulving (1983) who suggested that episodic memory was a uniquely human attribute and could not be possessed by animals. Although Tulving's view point was more tentative in 1983, by 1998 he stated that "episodic memory is not for the birds but for man" (Tulving & Markowitsch, 1998). They argue that animals are able to learn about the relationships between stimuli and events from specific episodes without having to encode temporal information that enables animals to locate these episodes in the past. Macphail (1982, 1998) held a rather sceptical view of the similarities between the memory processes of man and animals and presented arguments for doubting that animals possessed a sense of consciousness that allows them to mentally time travel, known as autonoetic consciousness. However as the world is full of unexpected dangers, *e.g.* predators, it could be considered beneficial for an animal to evolve in such a way that it develops a mechanism to enable the understanding of the world and its behaviours. Although this behaviour could be

considered to be implicit, it would be beneficial for the animal to encode, store and recollect information explicitly, *i.e.* the ability to identify a selection pressure and to react accordingly. He also argued that animals lack the knowledge of an overt language and due to the lack of agreed non-linguistic behavioural markers of conscious experience it makes it difficult to demonstrate episodic memory in animals. However one could argue that partaking and the subsequent recollection of an experience should be independent of the communication of the event to others, so knowledge of language should not be necessary for the possession of episodic memory.

Tulving's (1972) original definition stated that episodic memory 'receives and stores information about temporally dated episodes or events, and temporal-spatial relations among these events'. Therefore episodic memory combines information about the 'what' and 'where' of events as well as adding another dimension of 'when' the event occurred (temporally dated experiences). In episodic memory it is not only important that one is able to indicate a knowledge of the 'what', 'where' and 'when' but one must also be able to make a connection between the three features. This is particularly important as multiple events can occur in the same location but one must be able to separate each event according to 'what' happened and 'when'.

A landmark paper (Clayton & Dickinson, 1998) showed that scrub jays were in fact able to display the 'what', 'where' and 'when' of memory in an overt behavioural phenotype, in a phenomenon known as 'episodic-like' memory. Scatter hoarding birds scatter seeds across a large territory and rely on their memory to retrieve their hoards. Clayton and Dickinson's study utilised the food caching ability of scrub jays, which are

able to remember their cache sites and do not return to sites from which the food has already been retrieved.

In order to test whether the scrub jays had episodic-like memory the ‘degrade’ group were given perishable food (mealworms) and non-perishable food (peanuts) to cache in plastic, sand-filled ice-cube trays that served as individual caching sites. The scrub jays were given one type of food (*e.g.* mealworms), and another food (*e.g.* peanuts) 120 hours later to cache, followed by hoard retrieval 4 hours later. The birds showed a preference to cache, recover and eat perishable food while it was still fresh, and preferred mealworms to peanuts. If a long time had passed since their caching (124 hours) the scrub jays soon learned to ignore the mealworms and instead retrieve the non-perishable peanuts. If the food was presented in a different order, the scrub jays learned that the mealworms would still be fresh and therefore would search preferentially in the mealworm locations and ignore the peanut cache sites (Clayton & Dickinson, 1998).

Although the scrub jays in the ‘degrade’ group were able to recognise the trays in which they had cached the mealworms and peanuts earlier, it is possible that they had solved the task based on familiarity and had not necessarily remembered the ‘where’, ‘what’ and ‘when’ required for episodic-like memory. Therefore the performance of the ‘degrade’ group was compared to a control ‘replenish’ group which had never experienced degradation of their preferred choice of mealworms. As predicted, the scrub jays searched preferentially for the mealworms, regardless of when they had been cached, demonstrating that the scrub jays in the original ‘degrade’ group were not just searching the most familiar site. This series of experiments showed that the birds were

able to successfully remember the ‘what’, ‘where’ and ‘when’ of their food caching, thereby fulfilling Tulving’s criteria for episodic-like memory.

Bird *et al.* (2003) attempted to recreate Clayton and Dickinson’s scrub jay experiment in the common laboratory rat (Norway rat, *Rattus norvegicus*) in which rats were allowed to hide food items on an 8-arm radial maze (Olton and Samuelson, 1976). It had been shown previously that rats will carry items of food from the radial arms to produce a hoard at the centre of the maze (Whishaw And Tomie, 1989). The study by Bird *et al.* (2002) hypothesised that if rats could be persuaded to do the opposite and carry food from the centre of the radial maze to different arms then their memory for these storage locations could be assessed. Rats were placed within the centre of the radial maze and quickly learned to carry 4 pieces of food (cheese or pretzels) down the radial arms of the maze. Furthermore, rats also learned to retrieve the food items and, after a 1 min – 24 hour delay, showed a preference for returning accurately to arms in which they had stored food compared to arms where they had not. Like the scrub jays in Clayton and Dickinson’s study (1998) rats showed a food preference and searched selectively for cheese over pretzels, searching preferentially in the arms where they had previously stored cheese. In order to assess the ‘when’ component of episodic memory, Bird *et al.* (2003) attempted to teach the rats that cheese would be degraded after an hour, but edible 25 hours later. However unlike the scrub jays (Clayton and Dickinson, 1998), the rats failed to show any evidence for memory for ‘when’ they had hidden different types of food, as demonstrated by their inability to switch preference to pretzels during trials where the cheese had degraded. However the validity of this test can be questioned as a rat with intrinsic knowledge of degradation would know that it is nonsensical for food that is degraded after 1 hour to be edible after 25 hours. In the

study by Clayton and Dickinson (1998), the scrub jays were divided into 'degrade' and 'replenish' groups in which the latter group had never experienced degradation. It would perhaps have been more beneficial if Bird *et al.* (2003) had replicated this study more closely to more accurately measure the ability of the rats to display episodic-like memory.

## 3.4: Using the intrinsic novelty seeking behaviour of rodents to investigate memory

As mentioned above, Bird *et al.* (2003) were unable to demonstrate episodic-like memory in rats using a paradigm based in Clayton and Dickinson's scrub jay study (1998). However, the spontaneous novelty exploration paradigm has been successfully used to examine recognition memory in rats, and has been extended in order to assess episodic memory. These tests will be described in more detail in the following section.

### 3.4.1: Standard novel object recognition task

Ennaceur and Delacour published a landmark paper in 1988 describing a behavioural paradigm used to assess a rat's differential exploration of familiar and novel objects. Rats have an innate capacity to show preferential exploration of novel objects when compared to familiar objects, and this preference can be used as an index of memory. The standard novel object recognition task consisted of an exposure (sample) phase, a retention interval (between 1 minute – 24 hours), followed by a test phase (Figure 3.1A). During the sample phase, 2 identical objects ($A_1$ and $A_2$) were placed in the back corners of the arena. The rat was placed within the arena and exploration at each of the objects was assessed for 3 minutes after which the rat was returned to a separate holding

box. After a retention interval, the rat was returned to the testing box which contained an object that was identical to that seen previously ($A_3$) and a new object that the rat had not encountered before ($B_1$). The rat was considered to have 'remembered' the previously encountered objects if more time was spent exploring the novel object. As this test relies on the spontaneous exploration of novelty it does not require multiple training trials and therefore has no 'reference memory' component such as learning which alternatives belong to a rewarded set and those that are always unrewarded. In addition it is not based on positive or negative reinforcers such as food or electric foot shocks that could otherwise make the interpretation of the results difficult and misleading, as it is possible that reinforcers could modify the natural behaviour of the animal. Due to these reasons, this standard novel object recognition task designed by Ennaceur and Delacour (1988) has become a favoured method for assessing memory in rodents and has provided a useful basis for the testing of more complex forms of memory.

Research over the years has been to establish the differential roles of the hippocampus and the surrounding cortical regions in memory. The hippocampus, known to have a critical role in learning and memory, communicates with other brain regions via the fornix and via parahippocampal regions *e.g.* the perirhinal and postrhinal cortices. Various studies have assessed the effects of lesions in the perirhinal/ postrhinal cortices (Ennaceur *et al*., 1996; Bussey *et al*., 2000; Figure 3.1A), fornix (Ennaceur *et al*., 1996; Figure 3.1A) or hippocampus (Mumby *et al*., 2000 - Figure 3.1B; Langston and Wood, 2010 - Figure 3.1A) on performance in the spontaneous novel object recognition task (Ennaceur and Delacour, 1988). Results showed that although intact and fornix/ hippocampus lesion rats were able to correctly identify the novel object in this task

(Ennaceur *et al*., 1996; Bussey *et al*., 2000; Mumby *et al*., 2002, Langston and Wood, 2010), animals with perirhinal/ postrhinal cortex lesions (Ennaceur *et al*., 1996; Bussey *et al*., 2000) were impaired relative to control. Taken together, the findings of these studies have indicated a role of the perirhinal cortex, but not the hippocampus/ fornix in the standard novel object recognition task (Ennaceur *et al*., 1996; Bussey *et al*., 2000; Mumby *et al*., 2002, Langston and Wood, 2010). As the hippocampus and fornix are components of a single functional circuit, it is often assumed that damage at any point in the circuit should have similar effects on memory. Although some signals leave the hippocampus *via* the fornix, in rats and monkeys the hippocampal formation also sends major projections to the entorhinal cortex (Zola-Morgan *et al*., 1989). In addition, the entorhinal cortex enables the exchange of information between the hippocampus and the neocortex. Thus, it is important to remember that damage to the fornix does not necessarily impair information processing in the same way as direct damage to the hippocampus (Zola-Morgan *et al*., 1989).

The lack of impairment in animals with hippocampal/ fornix lesions in the standard novel object recognition task (Ennaceur *et al.*, 1996; Bussey *et al.*, 2000; Mumby *et al.*, 2002; Langston and Wood, 2010) indicates that, under many conditions, the hippocampus is not required for novel object recognition. However, it must be emphasised that it has been found that, at long delays, novel object recognition can be dependent on the hippocampus. Several studies have assessed the memory of rats with lesions of the hippocampus or fornix at 5 different delay intervals, ranging from 10 seconds to 24 hours (Clark *et al.*, 2000; Broadbent *et al*., 2004; Ainge *et al*., 2006). It was found that all the operated rats performed similarly to control rats at the shorter retention times (10 seconds and 1 minute), but were impaired with respect to control

animals across longer delays (10 minute, 1, 3 and 24 hours; Clark *et al.*, 2000; Broadbent *et al*., 2004; Ainge *et al*., 2006). During the trials with the longer retention intervals the animals are returned to their home cages. It is possibly the case that when the animals are given the opportunity to sleep between trials, the hippocampus plays a more important role in the novel object recognition task, therefore perhaps explaining the differences between the short- and long-term novel object recognition tasks following fornix/ hippocampal lesions. A 24 hour novel object recognition task will be used to assess the integrity of the hippocampus in the $Hdh^{Q111}$ mouse model of HD (section 6.2.3 and 6.6).

## 3.4.2: The 'what', 'where' and 'which' of episodic memory

Tulving's original (1972) definition focused on 3 key features of episodic memory, the 'what', 'where' and 'when'. Clayton and Dickinson (1998) stated that the 'when' component of episodic memory is particularly important: two individual episodes could potentially share the same 'what' and 'where' components, they cannot share the same 'when'. It has proved difficult to assess the ability of animals to keep track of time and limited evidence exists that animals are capable of using more than relative familiarity to judge how long ago an event occurred. However in response to Clayton's argument it is also possible to state that since any given episode cannot possibly have happened at the same time as another, it is therefore not necessary to encode an exact temporal marker for each event. As a result Eacott and Norman (2004) suggested that the 'when' part of Tulving's criteria may not be critical for the demonstration of episodic-like memory in animals. They instead developed a new triad for the criteria of episodic memory that consisted of 'what', 'where' and 'which', with the 'which' providing a novel contextual environment as a unique discriminator for events, *e.g.* what event

happened, where the event occurred, and any contextual information *e.g.* the weather. Eacott and Norman (2004) subsequently designed a task, based on Ennaceur and Delacour's (1988) standard novel object recognition task to examine a rat's ability to demonstrate memory for objects, their spatial position and the context in which they appeared, and showed that rats were in fact able to display memory for the new 'what', 'where' and 'which' triad.

In the study by Eacott and Norman (2004) rats were exposed to different objects ('what') in different locations ('where') in different testing boxes ('which'). In this task, the arena for the object-place-context task could be configured to provide 2 different contexts (X and Y). The object-place-context task consisted of 2 sample phases (sample 1 and sample 2) followed by a test phase (Figure 3.1I). In sample 1 the arena that was configured as Context X and contained 2 different objects ($A_1$ on the right and $B_1$ on the left). In sample 2 the arena was configured as Context Y and contained the objects seen in sample 1 but the object identity at each location was reversed ($B_2$ on the right and $A_2$ on the left). After a retention interval, the rat was placed back into the arena for the test phase in which the arena was configured as either Context X or Y, and contained 2 identical copies of one of the objects seen in the sample phases ($A_3$ and $A_4$ or $B_3$ and $B_4$). As a result one of the objects is in a novel configuration of place and context which has not been seen in either of the previous sample phases.

In addition, if the object-place-context task (Figure 3.1I) is a measurement of episodic-like memory then it could be predicted that performance in this task should be impaired by lesions within the hippocampal system. Subsequently, studies have assessed the effect of perirhinal/ postrhinal cortices or fornix/ hippocampal lesions on performance

in the object-place-context task (Eacott and Norman, 2004; Langston and Wood, 2010). Only rats with the fornix/ hippocampal lesions were impaired in their ability to integrate the 'what', 'where' and 'which' components required for episodic-like memory (Eacott and Norman, 2004; Langston and Wood, 2010). These studies therefore emphasise the need for the hippocampus in the integration of multiple features of an event. Specifically, it has been demonstrated that the encoding of object, place and context information is unaffected by lesions of the hippocampus, thereby inferring that it is the storage and retrieval of such information that is dependent on the hippocampus (Langston *et al*., 2010).

Although the integration of object, place and context configuration is required to display episodic memory, it is also important to determine that rodents are also capable of demonstrating memory for the individual components of episodic memory. The next section will therefore discuss the tests designed to assess memory for object in place (recognition that an object has swapped place with another object) and memory for object in context (recognition that a familiar object is in a different context to where it was previously encountered) (Eacott and Norman, 2004; Langston and Wood, 2010).

## 3.4.3: Extending novelty detection to look at memory for place and context

As stated previously the standard novel object recognition task was extended to include object-place memory (Eacott and Norman, 2004; Langston and Wood, 2010; Figure 3.1C). In the sample phase of the object-place task, 2 objects ($A_1$ and $B_1$) were located in the 2 far corners of the box. In the test phase, there were 2 copies of one of the objects seen in the sample phase (*e.g.* $A_2$ and $A_3$). Therefore in the test phase, one of the

objects was in a novel object-place configuration. Rats received lesions of the fornix/ hippocampus or were sham operated. In this object-place task both the sample and test phases occur in the same context and there is therefore no requirement to use context as a cue. All rats showed a preference for the novel object-place configuration indicating that the hippocampus is not required to process information about object-place using this protocol (Eacott and Norman, 2004, Langston and Wood, 2010).

However, this result is in contrast to the findings of 3 additional studies which tested rats with hippocampus/ fornix lesions in a test of location memory (Save *et al*., 1992; Ennaceur *et al*., 1997; Mumby *et al*., 2002) (Figure 3.1E and 3.1F). The results of these studies showed that intact animals showed preferential exploration at the object in the novel location, whereas rats that had received lesions of the hippocampus/ fornix failed to discriminate between the novel and familiar object (Save *et al*., 1992; Ennaceur *et al*., 1997; Mumby *et al*., 2002). The requirement for the hippocampus in these studies (Save *et al*., 1992; Ennaceur *et al.*, 1997; Mumby *et al.*, 2002) could be due to differences in experimental protocol when compared to the study by Eacott and Norman (2004) and Langston and Wood (2010). In the initial studies the test was an object-place task, with the objects appearing in the same locations in both the sample and test phases (Eacott and Norman, 2004; Langston and Wood, 2010). In contrast, the tests devised by Save *et al.* (1992), Ennaceur *et al.* (1997) and Mumby *et al.* (2002) were testing purely location memory, with the same objects being presented in different locations in the sample and test phases. It is perhaps the case that there is an element of spatial processing required to process information that an object is in a novel location, therefore recruiting the hippocampus. However in the case of the object-place tasks, the information that an object has swapped places can perhaps be supported by the surrounding cortical areas.

The lack of impairment following hippocampal lesions in the studies by Eacott and Norman's (2004) and Langston and Wood (2010) could also be due to the fact that the rats were always placed in the test arena in the same position, facing the same direction, therefore potentially allowing them to recognise each object-place configuration relative to their own starting position (Figure 3.1C). The previous studies (Ennaceur *et al*., 1997; Save *et al*., 1992; Mumby *et al.*, 2002) that have reported hippocampal deficits in location memory used multiple entry points, thereby promoting the use of an allocentric spatial framework to render the task hippocampal-dependent. In the study by Save *et al.* (1992), the entry points were randomised and differed for each trial, making it impossible to identify the novel location using an 'egocentric' approach (Figure 3.1F). Furthermore, in the tasks by Ennaceur *et al.* (1997) and Mumby *et al.* (2002), the rats were always placed in a position that was equidistant from both objects. However, in a task where one of the objects was placed in a novel location in the test phase, it follows that the point of entry was not consistent between each trial (Figure 3.1E). A further study (Langston and Wood, 2010) directly tested whether the object-place task could be forced to become hippocampal-dependent by introducing multiple entry points (Figure 3.1D). This study consisted of testing the animals in an 'allocentric' and 'egocentric' version of the object-place task. The procedure for the egocentric object-place task was the same as stated previously (Eacott and Norman, 2004; Langston and Wood, 2010) but for 2 of the test phases the rats entered the box from the east and were placed facing the east wall, and on the other 2 trials the rat entered the box from the west and were placed facing the west wall (Figure 3.1D). The entry point for the sample and test phases was therefore different for each trial, forcing the animals to produce an allocentric framework to solve the object-place task. Results showed that rats with

hippocampal lesions performed at chance levels on the allocentric object-place task in which different start points were used during the sample and test phases. When the same entry point for the sample and test phases was used (egocentric object-place task), both the control and hippocampal lesion group were able to correctly identify the novel object-place configuration relative to the sample phase. These data suggests that the object-place task is hippocampus dependent when an allocentric representation of spatial locations is required (*i.e*. the location task NOT the object place task) (Mumby *et al.*, 2002., Ennaceur *et al.*, 1997) but not when an egocentric strategy can be employed (Eacott and Norman, 2004; Langston and Wood, 2010).

Finally, the object-context task was designed in order to show that rodents were capable of demonstrating memory for context alone (Eacott and Norman, 2004; Langston and Wood, 2010). For this task each session was divided into 2 sample phases and a test phase (Figure 3.1G). Similar to the object-place-context task (section 3.4.2) the arena could be configured to provide 2 different contexts (X and Y). In sample 1, 2 identical objects ($A_1$ and $A_2$) were in Context X and in sample 2, 2 identical objects ($B_1$ and $B_2$) were in Context Y. The test phase was configured as either Context X or Y with copies of both the objects seen in the sample phases ($A_3$ and $B_3$). Therefore in the test phase one object was in a novel object-context configuration.

The effect of fornix/ hippocampal lesions on the object-context task was assessed in order to demonstrate that the impairment in the object-place-context task following hippocampal lesions was not secondary to an impairment in the memory for object and context (Norman and Eacott, 2005; Langston and Wood, 2010) (Figure 3.1G). Rats were given lesions of the fornix (Norman and Eacott, 2005), hippocampus (Langston

and Wood, 2010) or were sham-operated and were subsequently tested in the object-context task as described previously (Figure 3.1G). Both studies show that control animals were able to identify the novel object-context configuration in this task. Rats with fornix lesions were slightly impaired in their ability to identify the novel object-context configuration relative to sham operated controls but performed significantly above chance levels (Norman and Eacott, 2005) whereas animals with hippocampal lesions were unimpaired when compared to control animals (Langston and Wood, 2010). These data suggests that the fornix/ hippocampus are not necessary to process information about the context in which an object is encountered (Norman and Eacott, 2005; Langston and Wood, 2010).

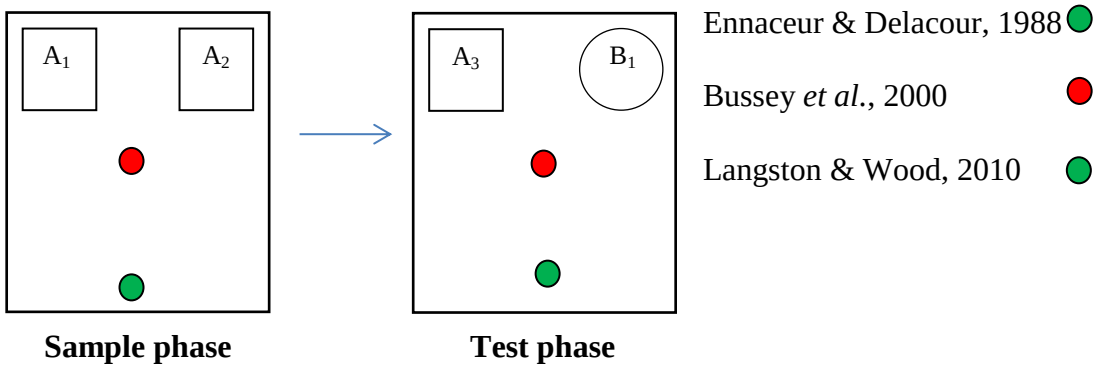
The findings of these studies are contradicted in a study by Mumby *et al.* (2002). However, the contrasting results could perhaps be explained by the differential protocols used in each study. As the context of the arena could be changed by inserting new floors and walls (Norman and Eacott, 2005; Langston and Wood, 2010), the arena in these studies occupied the same physical location in space and therefore had the same environmental cues (Figure 3.1G). In contrast, the object-context task performed by Mumby *et al.* (2002) involved 2 different contexts that were located in 2 different rooms (Figure 3.1H). The rooms differed in several ways, including the appearance of the ceiling and walls. Further cues were provided by strips of coloured cardboard which lined the top of the walls in one of the arena. The arenas were identical in every other way. Mumby *et al.* (2002) reported impairments in the object-context task following lesions of the hippocampus. The protocol differences in the current study when compared to previous studies (Norman and Eacott, 2005; Langston and Wood, 2010) in the context modification could therefore influence whether or not the object-context

task is hippocampus-dependent. The rats in the study by Mumby *et al.* (2002) did not have any cues within the test box and were instead required to use allocentric room cues. As stated previously, the study by Langston and Wood (2010) suggests that a task is rendered hippocampus dependent when an allocentric representation of spatial cues is required, perhaps providing an explanation for the deficit in object-context recognition in the study by Mumby *et al.* (2002).

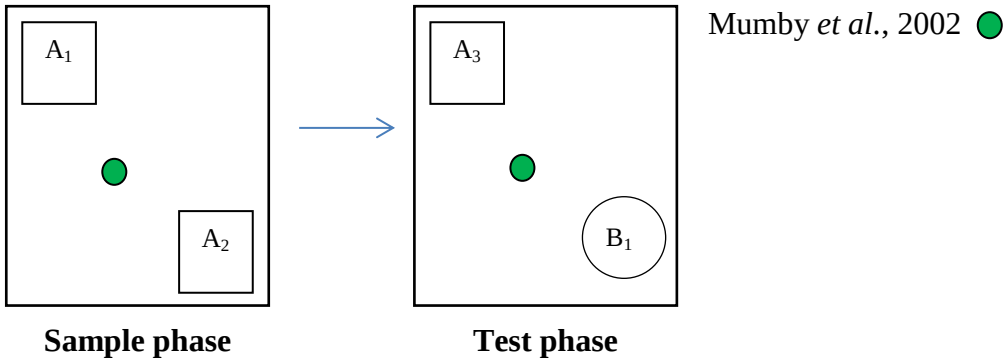
Impairments in short-/ long-term recognition and episodic memory have been demonstrated in human patients (see section 6.1.1) and mouse models of HD (see section 6.1.2). In order to assess episodic memory in the $Hdh^{Q111}$ mouse model of HD it is also necessary to assess the individual components of memory in order to demonstrate that any deficits in episodic memory are not secondary to impairments in the recognition of object-place or object-context (see section 6.2.1). As a further measurement of the integrity of the hippocampus in the early stages of HD, the long-term memory of the $Hdh^{Q111}$ mouse will be assessed in a hippocampus-dependent 24 hour novel object recognition task (see section 6.2.3).

## NOVEL OBJECT RECOGNITION

**A**



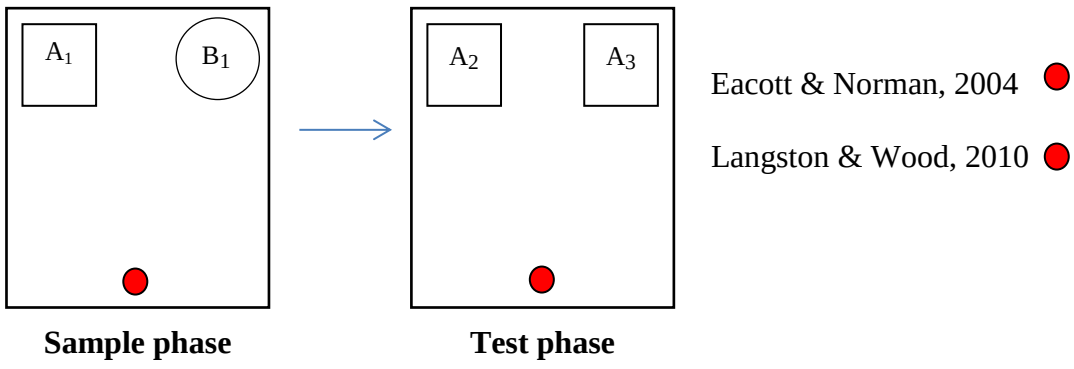

**B**



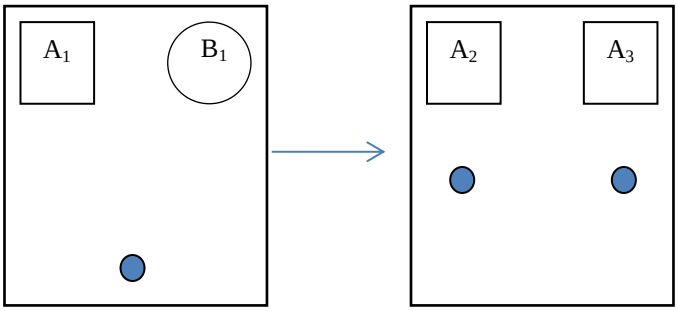

---

## OBJECT-PLACE RECOGNITION

**C**



**D**



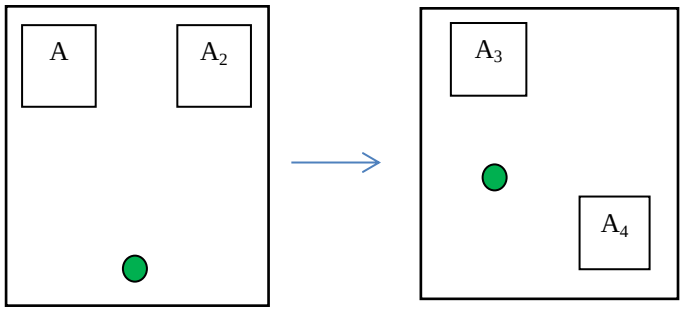

**Allocentric object-place**

Langston & Wood, 2010

---

## LOCATION MEMORY

**E**

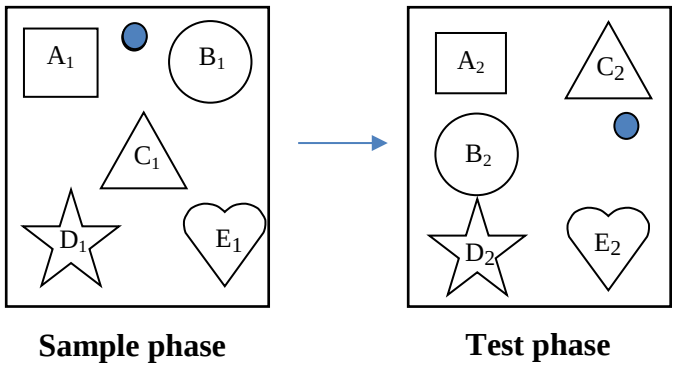



Dix & Aggleton, 1999

Ennacuer *et al.*, 1997

Mumby *et al.*, 2002

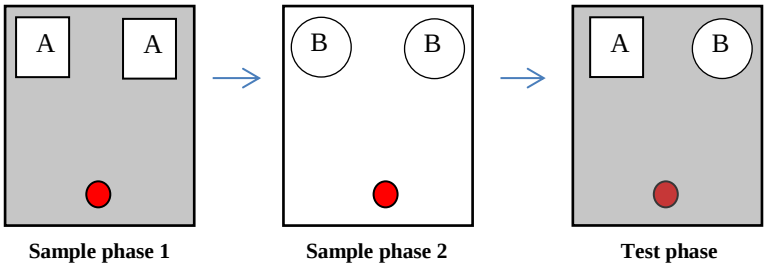
**F**




Save *et al.*, 1992

## OBJECT – CONTEXT RECOGNITION

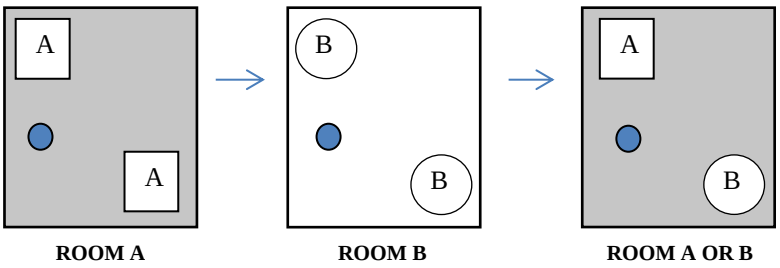
G




Langston & Wood, 2010

Norman and Eacott, 2005

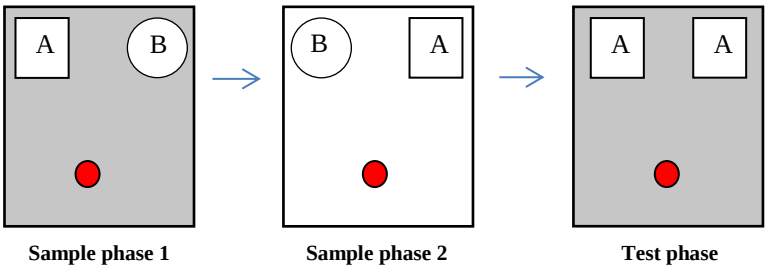
**H**




Mumby *et al.*, 2002

## OBJECT – PLACE – CONTEXT RECOGNITION

**I**




Eacott & Norman, 2004

Langston & Wood, 2010

***Figure 3.1**: **Schematic diagrams representing novel object, object place, location memory, object context and object-place-context tasks**. The corresponding references from the text are indicated. The coloured circles indicate the rodent entry point for each trial.*

# 4: γ-aminobutyric acid (GABA) and HD

## 4.1: Introduction

The cognitive deficits, in particular episodic memory, associated with human patients (see section 6.1.1) and mouse models of HD (see section 6.1.2) have been linked to abnormalities within the hippocampus (Rosas *et al.*, 2003). The $\alpha_5$-$GABA_A$ receptor is expressed primarily in the hippocampus, indicating a role in learning and memory (section 4.3). The following section will therefore provide an introduction to GABA and the $GABA_A$ receptor. The physiological role of the individual $GABA_A$ receptor subunits will be discussed, focusing particularly on $\alpha_5$-$GABA_A$ receptors. Furthermore, HD is associated with the major loss of striatal GABAergic medium spiny neurons (MSNs) (reviewed in Vonsattel and DiFiglia, 1998) and several studies have reported altered GABAergic synaptic transmission in mouse models of HD (Cepeda *et al*., 2004; Fujiyama *et al*., 2002; Cummings *et al*., 2009). The final section of this chapter will therefore discuss the role of $GABA_A$ receptors in HD (section 4.4).

## 4.2: GABA and the $GABA_A$ receptor

GABA is one of the main inhibitory neurotransmitters within the mammalian CNS. The $GABA_A$ receptor is a member of the Cys-loop family of ligand-gated ion channels, which also includes the nicotinic acetylcholine (nACh), glycine and 5-hydroxytryptamine ($5HT_3$) receptors. Arising from a common ancestral gene, the individual receptor subunits show amino acid sequence homology and possess several conserved features. Based on close analogy with the nACh receptor, it is thought that receptors in this family are comprised of 5 subunits arranged around a central ion-

conducting channel (Sieghart and Sperk, 2002; Unwin, 2005). Molecular cloning revealed the potential for a pronounced heterogeneity of $GABA_A$ receptors based on the existence of at least 19 subunit isoforms which are divided by their sequence identity into 3 main classes: $\alpha_{(1-6)}$, $\beta_{(1-3)}$, $\gamma_{(1-3)}$ and several more specialised forms: δ, ε, π, θ, and $\rho_{(1-3)}$ (Korpi *et al*., 2002; Rudolph and Möhler, 2006). $GABA_A$ receptors exist as heteromeric, pentameric complexes with a prevalent subunit ratio of 2α: 2β: 1γ (Farrar *et al*., 1999; Baumann *et al*., 2001; reviewed in Sarto-Jackson and Sieghart, 2008), with δ, ε and θ subunits occasionally substituting for the γ subunit (Nusser *et al*., 1996; Porcello *et al*., 2003; Sergeeva *et al*., 2005; reviewed in Sieghart and Sperk, 2002). Given the theoretical number of subunit combinations, multiple receptor isoforms are possible. However $GABA_A$ receptor heterogeneity is governed by spatial and temporal regulation of subunit composition and by rules that limit subunit assembly. Therefore, while receptors consisting of $\alpha_{(1-3)}$, $\beta_{2/3}$ and $\gamma_2$ are widely distributed throughout the CNS, other subunits ($\alpha_{(4-6)}$, δ, ε and θ) display a more limited pattern of expression (reviewed in Sieghart and Sperk, 2002). The $\alpha_1\beta_2\gamma_2$ combination represents the most abundant receptor subtype, but $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_x\gamma_2$ are also commonly expressed (reviewed in Sieghart *et al*., 1999; Möhler *et al*., 2002; Sieghart & Sperk, 2002).

## 4.2.1: Functional domains of $GABA_A$ receptors

$GABA_A$ receptor subunits exhibit a similar topology and consist of a long hydrophilic extracellular N-terminal region, 4 transmembrane helical segments (M1 – M4), a large intracellular loop between M3 and M4, and a short extracellular C-terminal domain (reviewed in Olsen and Sieghart, 2008; Figure 4.1).

The large extracellular N-terminal contains two cysteine residues that form a disulphide-linked loop which is common to all members of the Cys-loop superfamily. The extracellular segment also contains the GABA binding domain (Sigel, 2002; Kittler and Moss, 2003). By modelling the structure of the $GABA_A$ receptor on the basis of a snail acetylcholine binding protein (AChBP), the nACh receptor and information known on residues involved in the formation of the binding sites and subunit interfaces, it was determined that the subunits were arranged γ-α-β-α-β (Cromer *et al.*, 2002; Trudell, 2002). The GABA binding site is located in a pocket at the interface between each α subunit and the adjacent β subunit (for review see Sigel and Lüscher, 2011). The $GABA_A$ receptor has an anion selective pore, surrounded by an α-helical ring of 5 subunits (α, β, γ or ε, δ). There are 4 predicted membrane-spanning regions within each subunit (M1 - 4), and the M2 α-helices line the channel pore to form a conduction channel (Miyazawa *et al*., 2003). The transmembrane sections (M1 – 4) in $GABA_A$ receptors are connected by intracellular loops. The large intracellular loop connecting M3 – M4 contains sites for protein-protein interactions, phosphorylation and intracellular scaffolding proteins interaction sites (Kittler and Moss, 2003).

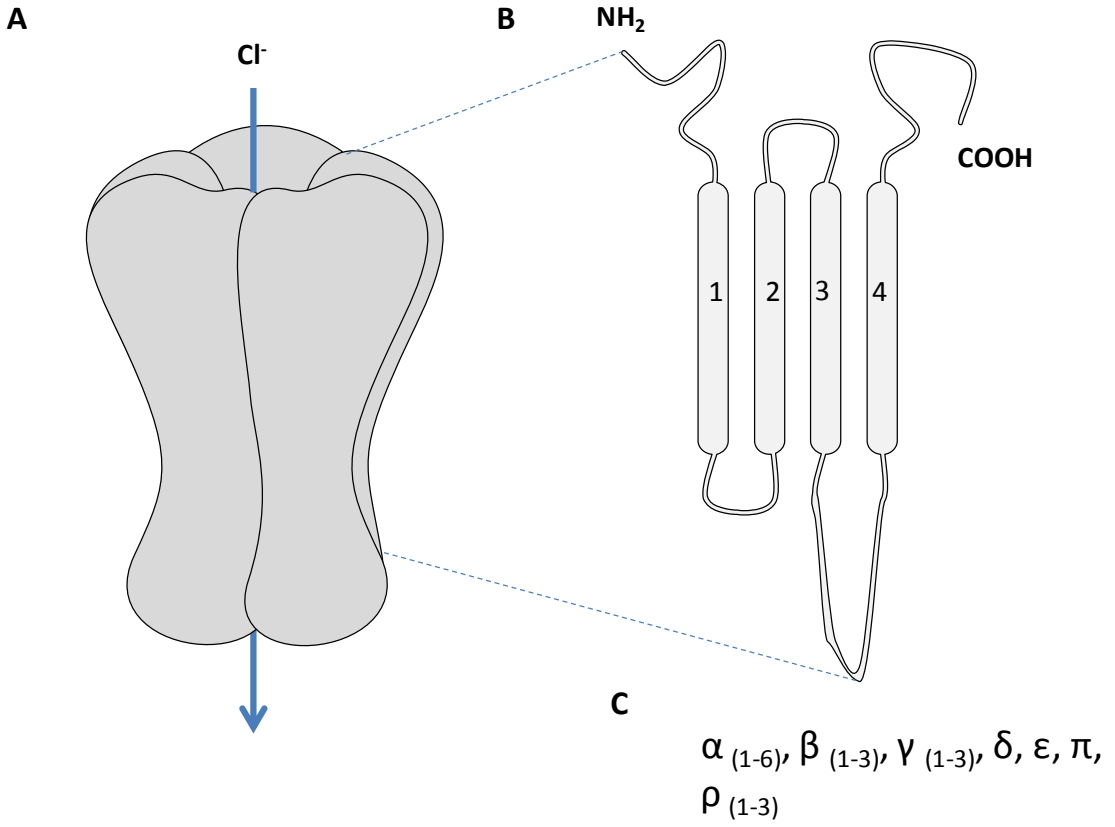



***Figure 4.1: Structure and heterogeneity of $GABA_A$ receptors.*** *A) Schematic representation of the predicted quaternary structure of $GABA_A$ receptors. B) Topological organisation of an individual subunit, illustrating the extracellular N-terminal domain, 4 transmembrane segments and the large intracellular loop between M3 – M4. C) Subunit repertoire of $GABA_A$ receptors.*

## 4.2.2: Physiological role of $GABA_A$ receptors

The subunit composition of $GABA_A$ receptors influence their physiological properties, their expression profile (which neurons are they expressed in and where: synaptic/extrasynaptic, presynaptic) and their pharmacological properties *e.g.* their interaction with benzodiazepines (Rudolph *et al.*, 1999; Low *et al*., 2000; McKernan *et al.*, 2000; Crestani *et al*., 2002; Yee *et al.*, 2005). While the majority of $GABA_A$ receptors (those containing $\alpha_1$-, $\alpha_2$-, $\alpha_3$-, or $\alpha_5$-subunits in combination with a $\gamma$-subunit) are benzodiazepine sensitive, there exists a minority of $GABA_A$ receptors ($\alpha_4$- or $\alpha_6$-subunit containing) which are insensitive to benzodiazepine due to the presence of an arginine *cf.* a histidine residue (found for $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits) at a key extracellular location of the subunit (Derry *et al*., 2004) This finding has been exploited to create "knock in" mice, engineered to be benzodiazepine insensitive (see below; Rudolph *et al*., 1999; Low *et al*., 2000; Crestani *et al*., 2002). Benzodiazepines act as allosteric modulators of $GABA_A$ receptors, enhancing the action of GABA by increasing the frequency of $GABA_A$ receptor channel openings (MacDonald and Olsen, 1994). Allosteric modulators such as benzodiazepines display a broad range of behavioural actions including: anxiolytic, sedation, perturbations of memory/cognition, analgesia, muscle relaxant and anticonvulsant properties (Savić *et al*., 2010). $GABA_A$ receptor knock-in mice have provided an important insight into the distinct physiological/ pharmacological roles contributed by individual $GABA_A$ receptor subtypes (reviewed by Möhler, 2006). This strategy was developed by generating 4 lines of point-mutated mice in which receptors containing the $\alpha_1$, $\alpha_2$, $\alpha_3$ or $\alpha_5$ subunits were rendered diazepam-insensitive by the introduction of a histidine to arginine point mutation in the drug-binding domain, while the physiological function of the receptor remained unaffected. Various benzodiazepine-induced behavioural responses from the wild type

and knock-in mice were then compared to clarify the *in vivo* relevance of the $GABA_A$ receptor subtypes. Consequently it has been established that the sedative component of diazepam is mediated through neuronal circuits that contain the $\alpha_1$-$GABA_A$ receptor (Rudolph *et al.*, 1999; McKernan *et al.*, 2000), whereas the anxiolytic activity is mediated through $\alpha_2$-$GABA_A$ receptors (Low *et al.*, 2000; Crestani *et al.*, 2002). The extent to which $\alpha_3$-containing receptors contribute to the anxiolytic effect of diazepam has yet to be established. Although the anxiolytic activity of diazepam was undiminished in mice that lacked the $\alpha_3$-$GABA_A$ receptor (Yee *et al.*, 2005), an inverse agonist at the $\alpha_3$-$GABA_A$ receptor was anxiolytic at high receptor occupancy (Dias *et al.*, 2005).

## 4.3: $\alpha_5$- containing $GABA_A$ receptors influence cognition

$GABA_A$ receptors that contain the $\alpha_5$-subunit have a relatively restricted distribution throughout the brain and are expressed primarily in the hippocampus, indicating a role in learning and memory. Although $\alpha_5$-$GABA_A$ receptors account for less than 5% of the total $GABA_A$ receptor population in the brain, in the hippocampus they represent 20% of all $GABA_A$ receptors further indicating a role for the $\alpha_5$-subtype in learning and memory processes (Sieghart, 1995).

A study by Collinson *et al.* (2002) demonstrated the role of the $\alpha_5$ subunit in learning and memory. Gene "knock-outs" or deletions of particular receptor subunits throughout the brain have proved useful in determining the roles of specific subunits, although compensatory mechanisms may complicate the interpretation of experiments utilising such mice. Homozygous mice were generated in which the $\alpha_5$ gene was deleted and such mice were subsequently tested for hippocampal function using tests of spatial

memory. In addition the performance of the mice in non-hippocampal-dependent learning tasks and anxiety tasks was also assessed. The mice were tested in the hippocampus-dependent 'matching-to-place' version of the MWM task. The results obtained showed a significant difference in the performance of the $\alpha_5$-knockout mice when compared to wild type, with the $\alpha_5$-knockout mice finding the platform significantly faster than the wild type mice (Collinson *et al.,* 2002). Differences in anxiety levels could potentially have a confounding effect on learning and memory in animals. In order to exclude this possibility the anxiety of the mice was tested on the elevated platform. The results showed that the $\alpha_5$-knockout mice did not appear to display any differences in background anxiety levels when compared to wild type, with both genotypes spending comparable times in the open and closed arms of the elevated platform (Collinson *et al.,* 2002). The results of this study therefore suggest that removal of $\alpha_5$-$GABA_A$ receptor expression results in disinhibition of the hippocampus, therefore promoting excitation and subsequently enhancing cognition.

In addition to the behavioural tests, *in vitro* brain slices were prepared from wild type and $\alpha_5$-knockout mice and the electrophysiological phenotype of the mice was assessed. To determine if the $\alpha_5$-knockout mice had alterations in synaptic function within the hippocampus, PPF and paired pulse depression (PPD) in the CA1 and DG was assessed. LTP was also examined in wild type and $\alpha_5$-knockout mice. Following paired-pulse stimuli, $\alpha_5$-knockout mice showed an increased facilitation of the fEPSP amplitude in the CA1 region of the hippocampus when compared to wild type mice. In contrast PPD in the DG remained unaffected, reflecting the reduced expression of $\alpha_5$-containing receptors in the DG when compared to the CA1 region of the hippocampus. In contrast to the enhancement of PPF in $\alpha_5$-knockout mice, there was no significant enhancement

of LTP following the application of a theta burst induction protocol. Whole-cell voltage-clamp recordings were also obtained from the CA1 neurons in the presence of glutamate and $GABA_B$ receptor blockers and the kinetics of the isolated inhibitory postsynaptic currents (IPSCs) were subsequently assessed. Although the deletion of the $\alpha_5$-subunit had no effect on the frequency, rise time and decay time kinetics, the peak amplitudes of the IPSCs were significantly smaller in the $\alpha_5$-knockout mice, consistent with the loss of this receptor subtype in the hippocampus. The results of this study by Collinson *et al.* (2002) further emphasised the role of the $\alpha_5$-$GABA_A$ receptor in learning and memory as performance in the hippocampal dependent MWM task was significantly improved in the $\alpha_5$-knockout mice, whereas the anxiety levels of the mouse remained unchanged. Studies suggested that alterations in the synaptic transmission could underlie the enhanced performance of the $\alpha_5$-knockout mice in the spatial memory task as IPSCs recorded from the $\alpha_5$-knockout mice exhibited reduced IPSC peak amplitude when compared to wild type. Previous studies have associated enhancements of learning and memory with increases in LTP (Tsien *et al*., 1996; Tang *et al*., 1999; see section 2.3). However, in the study by Collinson *et al.* (2002), LTP remained unaltered in the $\alpha_5$-knockout mice. In this case, the enhancement of the PPF alone could perhaps be sufficient to promote hippocampal memory, or other forms of synaptic plasticity could also be contributing to the enhanced cognition of the $\alpha_5$-knockout mice.

The role of the $\alpha_5$-subunit in memory was further demonstrated in a study by Crestani *et al.* (2002) where a point mutation (H105R) was introduced into the mouse $\alpha_5$-subunit gene. This knock-in mutation should have been silent, but immunohistochemical studies unexpectedly showed that the point mutation was associated with a specific reduction of hippocampal $\alpha_5$-$GABA_A$ receptors, whereas the pattern of distribution remained

unaffected (Crestani *et al*., 2002; Prut *et al*., 2010). The mice were subsequently tested on various behavioural tests to investigate the sedative, anticonvulsant and anxiolytic properties of diazepam (Crestani *et al*., 2002). The locomotor activity of the mice was tested in automated circular arenas subsequent to oral administration of diazepam. For anxiety, the effect of diazepam on the light-dark choice paradigm and the elevated plus maze was assessed. Fear conditioning was assessed by foot-shock studies. The investigation revealed that the behavioural responses to diazepam of both the wild type and $\alpha_5$ (H105R) point mutation mice were similar for the motor activity and locomotor activity tests. Furthermore, the anxiolytic action of diazepam remained unaltered in the $\alpha_5$ (H105R) point mutation mice as demonstrated by similar performances of both the wild type and knock-in mice in the elevated plus maze and in the light-dark choice test (Crestani *et al*., 2002).

The hippocampus plays an important role in specific types of associative learning and memory. Trace conditioning is a hippocampal dependent task in which the conditional stimulus and the unconditional stimulus are separated by a certain time interval (Crestani *et al*., 2002). However, when the 2 stimuli terminate together or overlap, *i.e.* in delay conditioning or contextual fear conditioning, the hippocampus is not required (Crestani *et al*., 2002). When first exposed to the tone and shock in the first learning session, both wild type and $\alpha_5$ (H105R) mice displayed similar levels of freezing in response to the shock. However, when exposed to the tone 48 hours later the $\alpha_5$ (H105R) mice showed an enhanced level of freezing when compared to the wild type mice, indicating that the $\alpha_5$-$GABA_A$ receptors are involved in associative memory (Crestani *et al*., 2002).

A further study by Prut *et al.* (2010) aimed to quantify the reduction of $\alpha_5$-$GABA_A$ receptor expression in the $\alpha_5$ (H105R) mice. In wild type brain slices, staining for $\alpha_5$-$GABA_A$ receptors showed moderate – strong staining along the hippocampal formation and parahippocampal sections. However, in the $\alpha_5$ (H105R) mice there was a 30% reduction in $\alpha_5$-$GABA_A$ receptor immunoreactivity in the hippocampus, although the layer and area-specific $\alpha_5$-subunit expression was retained (Prut *et al.*, 2010). Interestingly, $\alpha_5$-$GABA_A$ receptor staining in the neocortex and basal ganglia remained unchanged in the $\alpha_5$ (H105R) mice. As stated earlier, the $\alpha_5$ (H105R) mice demonstrated enhanced performance in the hippocampus-dependent trace fear conditioning task, providing further evidence for the involvement of $\alpha_5$-$GABA_A$ receptor in learning and memory (Crestani *et al*., 2002).

## 4.3.1: Synaptic *versus* extrasynaptic receptors

Among $GABA_A$ receptors in the brain, those that contain $\alpha_1$-, $\alpha_2$- and $\alpha_3$-subunits are normally present in the synapse (Nusser *et al*., 1996; Nyiri *et al*., 2001). These synaptic receptors are transiently activated by GABA and modulate 'phasic' inhibition. However, immunohistochemistry and *in situ* hybridisation studies have shown that $\alpha_5$-subunit containing receptors are primarily expressed in extrasynaptic locations on pyramidal neurons in the CA1 and CA3 regions of the hippocampus (Crestani *et al*., 2002; Brunig *et al*., 2002; Fritschy *et al*., 1998; Houser and Esclapez, 2003). Work from my host laboratory demonstrated that these extrasynaptic receptors are more sensitive to the actions of GABA, desensitise less and when activated by GABA, mediate a persistent 'tonic' form of inhibition (Caraiscos *et al*., 2004). The study revealed that the magnitude of GABA-mediated whole-cell currents of CA1 pyramidal neurons was greatly reduced for $\alpha_5$-knockout mice *c.f.* wild type indicating that the tonic current is

mediated through $\alpha_5$-$GABA_A$ receptors. In addition, there were no differences in the mIPSCs recorded from wild type and $\alpha_5$-knockout neurons, indicating that $\alpha_5$-$GABA_A$ receptors are not present at the synapse (Caraiscos *et al*., 2004).

As $\alpha_5$-$GABA_A$ receptors display preferential expression in the hippocampus and due to the association of reduced $\alpha_5$-$GABA_A$ expression and improved memory performance in behavioural studies (Collinson *et al*., 2002; Crestani *et al*., 2002), it follows that the tonic inhibition mediated by the $\alpha_5$-$GABA_A$ in the pyramidal neurons may play a key role in cognitive processes (Caraiscos *et al*., 2004). Long-term potentiation of synaptic efficacy following HFS of afferent pathways had long been associated with learning and memory (see section 2.3). However, as indicated above, LTP in CA1 neurons generated from $\alpha_5$-knockout mice does not differ from wild type neurons, although PPF was increased in $\alpha_5$-knockout slices (Collinson *et al*., 2002). The enhanced PPF was restricted to the CA1 of the hippocampus of $\alpha_5$-knockout mice (Collinson *et al*., 2002). PPF can be attributed to reduced inhibition by postsynaptic $GABA_A$ receptors and as mIPSCs are unchanged in $\alpha_5$-knockout mice this implies that the reduced tonic inhibition underlies the enhanced PPF (Caraiscos *et al*., 2004). Taken together, these results indicate that $\alpha_5$-$GABA_A$ receptor mediate tonic inhibitory conductance in hippocampal pyramidal neurons and may regulate memory (Chambers *et al*., 2002; Collinson *et al*., 2002; Caraiscos *et al*., 2004).

## 4.4: $GABA_A$ Receptors and HD

As stated above, HD is associated with the major loss of striatal GABAergic medium spiny neurons (MSNs; reviewed in Vonsattel and DiFiglia, 1998) and several studies have examined GABAergic transmission in mouse models of HD (Cepeda *et al*., 2004;

Fujiyama *et al*., 2002; Cummings *et al*., 2009). A study by Cepeda *et al.* (2004) used voltage-clamp recordings to examine GABAergic synaptic currents in MSNs in striatal slices prepared from 2 mouse models of HD. Experiments were carried out on 3 age groups of the R6/2 mouse; pre-symptomatic animals (3 weeks old), when overt symptoms begin (5 – 7 weeks old) and when full behavioural phenotype has been expressed (9 – 14 weeks; for more phenotypic detail of the R6/2 mouse see section 1.2.1.1). Examination of the spontaneous GABAergic synaptic currents demonstrated that, by 5 – 7 weeks, R6/2 mice showed a significant increase in the mean frequency of spontaneous GABAergic currents when compared to wild type mice. These data suggests that striatal GABAergic interneurons fire more frequently in the transgenic mice. Following treatment with TTX, GABAergic currents were similar to control, suggesting that the increase in frequency of GABA synaptic activity in the R6/2 mouse is dependent primarily on action potential generation. Furthermore, analysis of the sIPSCs in the 9 – 14 week age group showed significant faster rise and decay kinetics suggesting changes to the postsynaptic $GABA_A$ receptors. Studies with immunofluorescence indicated that staining for the $\alpha_1$-$GABA_A$ receptor was significantly increased in 12 week old R6/2 mice, perhaps explaining the faster kinetics and decay times observed in the MSNs from R6/2 mice. These studies indicate that changes in $GABA_A$ receptor function may contribute to HD. Several other studies have also observed an up-regulation of $GABA_A$ receptor expression, specifically an increase in $\beta_{2/3}$-$GABA_A$ receptor expression in the substania nigra in mouse models (Fujiyama *et al*., 2002) and an increase in $\alpha_1$, $\beta_{2/3}$ and $\gamma_2$-$GABA_A$ receptor expression in the globus pallidus of human patients with HD (Thompson-Vest *et al*., 2003; Allen *et al*., 2009).

The study by Cepeda *et al.* (2004) demonstrated that the frequency of IPSCs increased in striatal MSNs from R6/2 mice. As the cerebral cortex provides the main excitatory drive to the striatum (Markram *et al*., 2004), a follow up study in 2009 (Cummings *et al*., 2009) examined synaptic currents within cortical pyramidal neurons of R6/2, YAC128 and CAG140 mouse models in order to gain a better understanding of how neurons are affected in HD (for more details on mouse models see section 1.2). Using whole-cell voltage-clamp techniques, it was established that, in common with MSNs of the striatum (Cepeda *et al*., 2004), the frequency of spontaneous IPSCs was also increased in the cortical pyramidal neurons of pre-symptomatic mouse models of HD. This increased frequency was sensitive to treatment with TTX, suggesting a presynaptic action, perhaps resulting from an increased action potential discharge or an increase in the probability of release or number of release sites. However, as no differences in the paired-pulse ratio were evident, it was speculated that the firing properties of GABAergic interneurons are altered in mouse models of HD, subsequently increasing inhibition in pyramidal neurons (Cummings *et al*., 2009). An increase in postsynaptic receptors was considered unlikely as there was no change in the amplitude of mIPSCs, or of the GABA-evoked currents recorded from dissociated neurons derived from such mice. A reduction in the frequency of IPSCs, that was insensitive to TTX and therefore independent of action potential firing, was observed in post-symptomatic R6/2 mice. Although the results indicated an increased probably of release, this was confounded by a depletion of available GABA-containing vesicles within the synaptic terminals, perhaps underlying the decrease in sIPSC frequency in symptomatic models of HD (Cummings *et al*., 2009).

## 4.4.1: $GABA_A$ receptor trafficking and the regulation of synaptic strength

Synaptic inhibition plays an important role in regulating neuronal excitability and information processing in the brain. The number of $GABA_A$ receptors present at the plasma membrane is a critical determinant of inhibitory synaptic strength. Alterations of the excitatory/ inhibitory balance may lead to changes in neuronal excitability and/or disrupted memory processing (Jacob *et al.,* 2008). In addition to the regulation of apoptosis and transcription, huntingtin may also have a neurotoxic role in HD by altering the intracellular transport of proteins including the NMDA receptor (Fan and Raymond, 2007). $GABA_A$ receptor function may also be impaired in HD. Following endocytosis, the fate of $GABA_A$ receptors may be subject to regulation by an interaction of the receptor with huntingtin-associated protein (HAP1), a binding partner of Huntingtin (Duyao *et al*., 1993; Li *et al*., 1995). HAP1 inhibits the degradation and facilitates the recycling of $GABA_A$ receptors following endocytosis, increasing the number of $GABA_A$ receptors expressed at the cell surface and synaptic receptor number. Therefore HAP1 may play a critical role in controlling fast synaptic inhibition by regulating the membrane trafficking of internalised $GABA_A$ receptors, leading to compromised inhibition and disruption of the excitatory/ inhibitory balance (Kittler *et al*., 2004). The next section will introduce HAP1 and discuss its role in $GABA_A$ receptor trafficking.

## 4.4.2: HAP1 expression and function

HAP1 is expressed predominantly within the CNS, especially in the forebrain, cerebral cortex and the cerebellum (Li *et al.,* 1995). HAP1 is a cytoplasmic protein and associates with microtubules and membranous organelles *e.g.* mitochondria,

endoplasmic reticulum (Martin *et al.,* 1999). The localisation of HAP1 and Huntingtin is similar which suggests that they both have a role in intracellular transport (Gutekunst *et al.,* 1998).

HAP1 acts as a scaffold protein, enabling the packaging of various proteins for transport along the microtubules. HAP1 acts as one of the components of cargo-motor molecules and participates in intracellular trafficking (Li & Li, 2005). HAP1 may regulate the turnover and stabilisation of membrane receptors at the cell surface to maintain neuronal responses to neurotransmitters by increasing expression of membrane receptors by inhibiting degradation *via* lysosomal pathways and enhancing recycling pathways. In addition, HAP1 has been shown to bind the β subunits of $GABA_A$ receptors (Kittler *et al.,* 2004). Under basal conditions, synaptic $GABA_A$ receptors undergo clatherin-dependent endocytosis, and the internalised receptor is subsequently recycled back to the membrane surface or targeted for lysosomal degradation. HAP1 inhibits receptor degradation, in turn facilitating the recycling of receptors back to the membrane. The overexpression of HAP1 results in an increase in $GABA_A$ receptor surface number, thereby increasing neuronal excitability (Kittler *et al*., 2004).

### 4.4.3: HAP1 mediates delivery of $GABA_A$ receptors to the plasma membrane

In order to understand the role of synaptic $GABA_A$ receptors in the regulation of inhibitory synaptic transmission, it is important to understand the molecular machinery that delivers the receptors to the synapses. It was recently established (Twelvetrees *et al.,* 2010) that HAP1 acts as an adapter, linking $GABA_A$ receptors to the kinesin family of motor protein 5 (KIF5), forming a motor complex for rapid delivery of $GABA_A$

receptors to synapses. Furthermore, mutant Huntingtin disrupts the formation of this complex, inhibiting KIF5-dependent $GABA_A$ receptor trafficking (Gauthier *et al.,* 2004; Li *et al.,* 1995). In electrophysiological studies utilising the whole-cell voltage-clamp technique, cortical neurons (maintained in cell culture) were dialysed, *via* the patch pipette, with an antibody (SUK4) to block KIF5 motor protein activity. When the activity of the KIF5 motor protein was inhibited, the amplitude of mIPSCs decreased, but mIPSC kinetics were not affected, suggesting that KIF5 does not selectively transport $GABA_A$ receptors to the receptor with particular kinetics (Twelvetrees *et al.*, 2010). It was hypothesised that the effect of SUK4 on inhibitory transmission was mediated by blocking KIF5-dependent $GABA_A$ receptor delivery to synapses. In order to test this, SUK4, or a control antibody, was introduced into neurons maintained in cell culture and the effect on $GABA_A$ receptor clustering was determined. Neurons treated with SUK4 showed a decrease in the $GABA_A$ receptor cluster area in dendrites when compared to mock-treated neurons, suggesting that blocking KIF5 activity decreases the delivery of $GABA_A$ receptors to the membrane (Twelvetrees *et al*., 2010).

The expanded glutamine repeats present during HD enhance the function and trafficking of the NMDA receptor and it has been proposed that this effect exacerbates excitotoxicity (Fan *et al.,* 2007). In addition, increased neurotransmission and neuronal excitability are also proposed to contribute to the neuronal degradation caused by the full-length polyglutamine huntingtin protein early in pathology (Romero *et al.,* 2008). It has been suggested that NMDA receptors are transported by a second kinesin motor protein, KIF17 (Guillaud *et al*., 2003). When SUK4 was dialysed into the patch-pipette, although the $GABA_A$ receptor-mediated responses were decreased, the NMDA

receptor-mediated responses remained unaffected, suggesting a specificity of KIF5 for selectively transporting $GABA_A$ receptors to synapses (Twelvetrees *et al.*, 2010).

Immunoprecipitation experiments were performed in rat brain lysate to show that KIF5 could readily be immunoprecipitated with $GABA_A$ receptors, indicating that KIF5 motor proteins are closely associated with the $GABA_A$ receptor *in vivo* (Twelvetrees *et al.*, 2010). It had already been established that HAP1 function is disrupted by the mutant polyglutamine version of huntingtin that causes HD (Gauthier *et al.*, 2004., Li *et al.*, 1995) suggesting that HAP1-KIF5-dependent trafficking could therefore be a likely target for disruption in HD, leading to pathological alterations in inhibition. To further investigate this possibility, live cell-imaging experiments were used in neuronal cells derived from homozygous knock-in mice in which an expanded glutamine repeat (109 CAG) had been inserted into the endogenous mouse huntingtin gene (Trettel *et al.*, 2000). It was therefore possible to track the movement of vesicles and to demonstrate a significant decrease in the velocity of $GABA_A$ receptor transport vesicles. This observation suggested that mutant huntingtin decreased the accumulation of $GABA_A$ receptors at inhibitory synapses by altering the trafficking ability of HAP1-KIF5-dependent $GABA_A$ receptor transport vesicles for receptor delivery to inhibitory synapses (Twelvetrees *et al.*, 2010). In agreement, electrophysiological studies in transfected cortical neurons showed that, when compared to wild-type huntingtin transfected cells, expression of mutant huntingtin with an expanded polyglutamine repeat showed a significant decrease in the mIPSC amplitude (Twelvetrees *et al.*, 2010). A recent study by Yuen and colleagues (2012) was performed to determine whether impaired synaptic inhibition in the N171-82Q (82 CAG repeats) mouse model of HD results from the loss of $GABA_A$ receptor transport due to the disruption of the

HAP1/KIF5/$GABA_A$ receptor complex *in vivo.* Results showed that, although synaptic inhibition was normal in pre-symptomatic mice, the amplitude of miniature IPSC was significantly decreased in both cortical and striatal neurons from post-symptomatic N171-82Q mice. In addition paired pulse ratios and IPSC frequencies were unchanged, indicating the depression of GABAergic functions in the N171-82Q mice is due to altered postsynaptic $GABA_A$ receptors and not caused by changes to presynaptic GABA release (Yuen *et al*., 2012). In agreement, it was demonstrated that cell surface $GABA_A$ receptor expression is reduced in symptomatic mice. It is known that HAP1 interacts with $GABA_A$ receptors (Kittler *et al*., 2004) and KIF5 (Twelvetrees *et al*., 2010), and due to the fact that mutant huntingtin binds to HAP1 with a greater affinity than to wild type huntingtin (Li *et al*., 1995), Yuen and colleagues (2012) speculated that kinesin-dependent $GABA_A$ receptor transport could be impaired in the N171-82Q mice due to abnormal mutant huntingtin/ HAP1 interactions. This hypothesis was confirmed when it was demonstrated using co-immunoprecipitation that the expanded polyglutamine repeats in N171-82Q mice caused the dissociation of the KIF5/$GABA_A$ receptor complex from the microtubules and the dissociation of $GABA_A$ receptors from KIF5, thus providing a possible mechanism for disrupted $GABA_A$ receptor transport in HD (Yuen *et al*., 2012).

Finally, in addition to altered GABAergic inhibition, NMDA receptor function is also reported to be abnormal in striatal neurons of mouse models of HD (Zeron *et al.,* 2002; Fan *et al*., 2007) More specifically, extrasynaptic NMDA receptors have been implicated in HD (Okamoto *et al*., 2009; Milnerwood *et al*., 2010). Differential roles for NMDA receptors have been uncovered depending upon their location (Papadia and Hardingham, 2007). Synaptic NMDA receptors activate cellular survival pathways

while extrasynaptic receptors activate apoptotic pathways, and an imbalance of activity or number of each type of NMDA receptor would therefore influence cell survival or cell death. Subsequently, Milnerwood *et al* (2010) demonstrated that this synaptic/ extrasynaptic balance is disrupted, with increased extrasynaptic NMDA receptor activity present in MSNs in the YAC128 mouse model of HD. It was determined that the increased activity of the extrasynaptic NMDA receptor required capase-6 cleavage, a step critical for the production of toxic huntingtin fragments (Milnerwood *et al*., 2010). Similarly, an additional study noted that activation of synaptic NMDA receptors resulted in an increased resistance to cell death due to the formation of huntingtin inclusions. On the other hand, activation of extrasynaptic NMDA receptors reduced the formation of huntingtin inclusions, thereby increasing the vulnerability of mutant huntingtin expressing cortical neurons (Okamoto *et al*., 2009).

Collectively, the above data suggests that altered KIF5 motor dependent-trafficking may directly contribute to reduced synaptic inhibition and altered information processing in HD. NMDA receptor function may also be altered, perhaps contributing to dysfunction of the excitatory/inhibitory balance (Yuen *et al*., 2012). It is therefore an interesting possibility that mutant Huntingtin could influence GABAergic neurotransmission and synapse development leading to disrupted $GABA_A$ receptor trafficking and altered synaptic inhibition and subsequently an enhanced neuronal excitability, contributed also by impaired NMDA receptor function, in HD. Of relevance to the current study, the impact of HD on the expression of extrasynaptic $\alpha_5$-$GABA_A$ receptor expression is not known.

# 5: Experiment 1: Characterisation of the motor phenotype of the $Hdh^{Q111}$ mouse model of HD.

## 5.1: Introduction

In humans, HD is diagnosed following the appearance of an overt motor phenotype (Sturrock and Levitt, 2010; see section 1.1.3). In addition, motor deficits are also evident in several mouse models of HD (Mangiarini *et al*., 1996; Carter *et al*., 1999; Lüesse *et al*., 2001; Wheeler *et al*., 2000; Wheeler *et al*., 2002; Menalled *et al*., 2003; Hickey *et al*., 2005; Stack *et al*., 2005; Milnerwood *et al*., 2006; Heng *et al*., 2007; Gray *et al*., 2008) (see section 1.2). In order to replicate the motor deficits seen in human patients, the following section of this thesis will assess the motor phenotype of the $Hdh^{Q111}$ mouse model of HD. Although motor deficits are not as pronounced in knock-in models when compared to other transgenic models, careful testing using refined test settings can be used reveal early deficits. The slow progression of the disorder in these models allows the evaluation of the early changes likely to be the most important to target therapeutically in order to prevent further progression of the disease. Thus the $Hdh^{Q111}$ knock-in mice offer an ideal model in which behavioral testing can be used to evaluate new therapeutic approaches designed to delay the onset of HD.

In an attempt to characterise the motor phenotype of the $Hdh^{Q111}$ mouse model of HD, the rotarod was used to assess motor co-ordination (2 - 12 months) while the circular running tracks and the activity box were used to assess locomotor activity (both tested at 13 months).

# 5.2: Methods

## 5.2.1: Breeding and genotyping

The phenotype of the $Hdh^{Q111}$ mouse model of HD was assessed in this thesis. Homozygous $Hdh^{Q111}$ ($Hdh^{Q111+/+}$) mice were purchased from the Jackson Laboratory, maintained in the laboratory as an inbred colony and used, in combination with wild type (WT) C57/BL6 mice, to produce heterozygous $Hdh^{Q111}$ ($Hdh^{Q111+/-}$) mice. Note, in all the subsequent results chapters, genotypes were evaluated using the NucleoSpin Tissue DNA purification kit (Macherey-Nagel) for DNA isolation and the Hot star TaqPlus polymerase kit (Qiagen) for DNA amplification. Agarose gels were used to separate the proteins showing a WT Huntingtin protein band at 424 basepairs and a knock-in Huntingtin protein band at 694 basepairs (Figure 5.1).

## 5.2.2: Rotarod

The rotarod has been specifically designed for making automated measurements of motor co-ordination in rodents and is one of the most commonly used tests of motor function in mice (Dunham and Miya, 1957; Jones and Roberts, 1968) (Figure 5.2). The rotarod (Ugo Basile, Italy) consists of horizontal beams with dividers to separate multiple mice being tested concurrently. Mice were tested on the accelerating rotarod paradigm (2 – 12 months). The mice were tested on the first week of every month: 1 training day to allow acclimatisation to the rotarod apparatus, followed by 3 trials on 3 consecutive days. During the test period, each mouse was placed on the rotarod with increasing speed, from 6 rpm to a maximum speed of 50 rpm, for a maximum time of 10 minutes. A trip switch was located beneath the rod and recorded the latency until the mouse fell from the rotating rod.

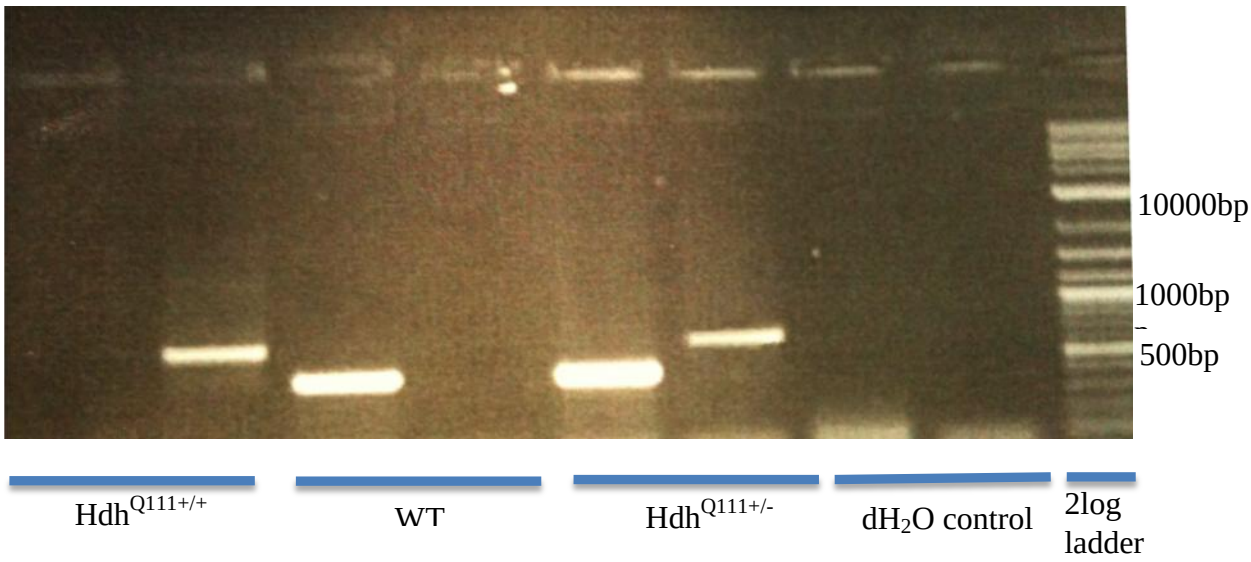



***Figure 5.1: Representation of genotyping results.***

*Agarose gels were used to separate the proteins. WT mice show 1 WT band (424 base pairs). Hdh$^{Q111+/+}$ mice display 1 knock-in band (694 base pairs). Hdh$^{Q111+/-}$ mice show 1 WT band and 1 knock-in band.*


***Figure 5.2: Photograph of rotarod apparatus.***

*The mice were placed on the accelerating rotarod (6 rpm – 50 rpm; maximum time of 10 minutes). A trip switch was located beneath the rod and recorded the latency until the mouse fell from the rotating rod.*

## 5.2.3: Circular runways

Locomotor activity was assessed in individual polypropylene circular runways (diameter = 245 mm, corridor width = 65 mm) (13 months) (Figure 5.3). Mice were tested on 4 consecutive days. The runways were transected by eight photobeams spaced at regular intervals and forward locomotion was defined as consecutive breaks of adjacent beams. Photobeam breaks were counted in 30 second time samples, at 4 minute intervals, and data was collected by an attached PC via a Med-PC interface (Med Associates, WT, USA) (Dixon *et al*., 2010).

## 5.2.4: Activity Box

The locomotor activity of mice was also measured using an Activity Monitor (Benwick Electronics, Norfolk, UK) using infrared beams to detect movement (13 months) (Figure 5.4). Mice were tested on 4 consecutive days. The mice were placed in a Perspex chamber (32 x 20 x 19 cm) in which two sets of beams were arranged in a grid formation covering the length and width of the chamber. The lower beams (2 cm above the floor) could detect the animals' horizontal movement and the higher beams (7 cm above the floor) could detect any rearing. Movement was measured by beam breaks and recorded as counts of activity. A mouse was considered mobile if the movement of the animal broke two consecutive beams but not if the same beam was broken twice. The animal was placed in the activity box for 10 minutes over a series of 4 days and the locomotor activity was logged at the end of each 10 minute session. Habituation was said to have occurred when the rodent became familiar to the environment, as demonstrated by reaching a constant but reduced level of activity between sessions. This will occur if the animals have memory of the previous exposures to the testing environment.

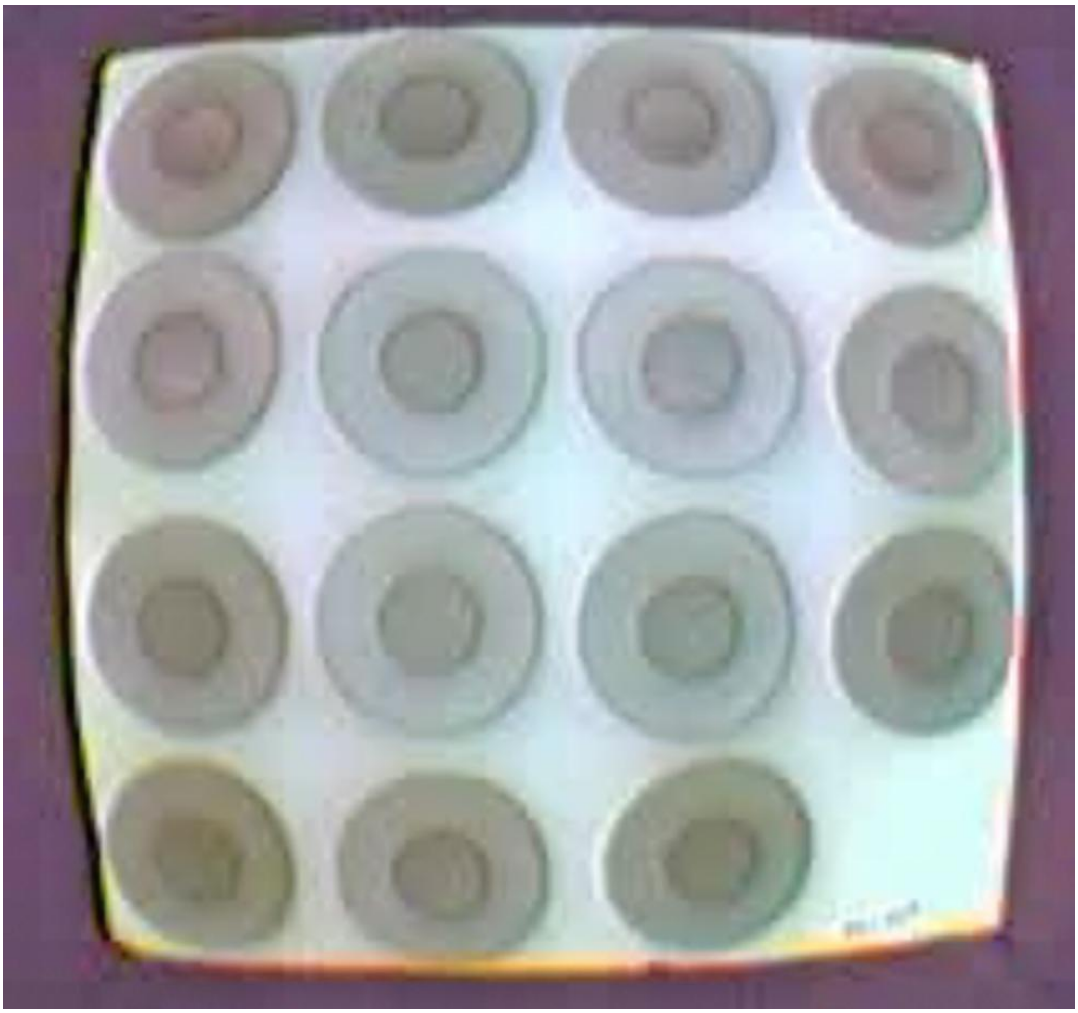


***Figure 5.3: Photograph of the circular runway apparatus.***

*Each circle represents an individual polypropylene circular runway. The runways were transected by photobeams; forward locomotion was defined as consecutive breaks of adjacent beams.*

***Figure 5.4: Photograph of the activity box apparatus.***

*The mice were placed in the activity box (10 minutes, 4 consecutive days). Lower beams detected horizontal movement and higher beams detected rearing. Movement was measured by beam breaks and recorded as counts of activity.*

## 5.3: Results: rotarod

In order to determine whether the presence of the mutant *Huntingtin* gene resulted in the generation of any motor deficits, WT ($n$ = 9), $Hdh^{Q111+/+}$ ($n$ = 10) and $Hdh^{Q111+/-}$ ($n$ = 11) were tested on the accelerating rotarod apparatus (2 – 12 months). Note that 1 $Hdh^{Q111+-}$ mouse died before the end of the 12 months so was not included in the analysis.

### 5.3.1: $Hdh^{Q111}$ mice show no progressive weight loss

Mice were weighed at the beginning of each of their monthly rotarod trials. WT mice displayed a gradual weight increase from 3 months (26.9 ± 0.7 g) to 12 months (33.5 ± 0.4 g; Figure 5.5A). $Hdh^{Q111+/+}$ mice increased in weight from 26.2 ± 0.6 g at 3 months to 32.7 ± 0.5 g at 12 months, $Hdh^{Q111+/-}$ mice increased from 25.3 ± 0.4 g to a final weight of 32.2 ± 0.6 g at 12 months ($f_{(2, 29)} = 1.222$; $P > 0.05$). The weights of the mice were compared using a repeated measures ANOVA with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and age (3 - 12 months) as the within subjects factor. Results indicated a significant effect of age ($f_{(9, 18)} = 271.474$, $P < 0.05$), but no significant effect of genotype ($f_{(2, 26)} = 2.491$, $P > 0.05$) or age *vs.* genotype interaction ($f_{(18, 234)} = 1.205$, $P > 0.05$). These data show that the presence of the mutant *Huntingtin* gene does not result in any discrepancies in weight between the genotypes up to the age of a year.

### 5.3.2 : $Hdh^{Q111}$ mice show a mild motor phenotype on the rotarod

Mice were tested using the accelerating rotarod paradigm. The data was initially analysed in the raw form (Figure 5.5B). A repeated measures ANOVA was performed with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and age (2 - 12 months) as the within subjects factor. Results indicated no significant effect of

genotype ($f_{(2, 26)} = 1.332$, $P > 0.05$), a significant effect of age ($f_{(10, 260)} = 4.033$, $P < 0.05$) and an age *vs.* genotype interaction ($f_{(20, 260)} = 2.385$, $P < 0.05$). Bonferroni corrected pair-wise comparisons between groups confirmed that $Hdh^{Q111+/+}$ mice, but not $Hdh^{Q111+/-}$ mice, showed an increased latency to fall from the beam when compared to WT mice (WT: 138.8 ± 11.1 sec; $Hdh^{Q111+/+}$: 274.1 ± 33.5 sec; $Hdh^{Q111+/-}$: 239.4 ± 33.2 sec; $P < 0.05$) at 2 months. At 3 months, both $Hdh^{Q111+/+}$ (253.8 ± 41.2 sec) and $Hdh^{Q111+/-}$ (235.7 ± 18.8 sec) mice showed increased latency to fall from the rod when compared to WT mice (148.5 ± 14.4 sec; $P < 0.05$). For all of the subsequent months there were no differences between the performances of the three genotypes (all $P > 0.05$).

In order to further analyse the data the raw data was normalised to show the progression of the individual genotypes as they aged (Figure 5.5C). A mean latency to fall was calculated for each genotype at 2 months and for the following months each mouse was then plotted as a percentage of the original value at 2 months. A repeated measures ANOVA showed a significant effect of genotype ($f_{(1, 26)} = 6.622$, $P < 0.05$), no significant effect of age ($f_{(10, 260)} = 1.178$, $P < 0.05$) and an age *vs.* genotype interaction ($f_{(20, 260)} = 1.763$, $P < 0.05$). Bonferroni corrected pair-wise comparisons indicated that the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice was consistent up to the age of 5 months (all $P > 0.05$). At 6 months $Hdh^{Q111+/+}$ ($P < 0.05$) but not $Hdh^{Q111+/-}$ ($P > 0.05$) mice spent significantly less time on the rod when compared to their performance at 2 months. Both $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice show impairments by 7 months ($P < 0.05$). Bonferroni corrected pair-wise comparisons between groups confirmed that both the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice show motor co-ordination deficits on the rotarod at 8, 11 and 12 months, when compared to their performance at 2 months (all $P < 0.05$), whereas

only $Hdh^{Q111+/+}$ mice show impairments at 9 and 10 months ($P < 0.05$). The performance of WT did not change over the course of the testing period ($P > 0.05$). The performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice is similar at all ages ($P > 0.05$). These data show that $Hdh^{Q111+/+}$ mice show impaired performance in the accelerating rotarod task by 6 months. $Hdh^{Q111+/-}$ mice show deficits from 7 months, although this is not consistent at all the time points and would therefore perhaps advantageous to test a larger sample of mice. Additionally, the decreased locomotion on the rotarod over time is somewhat complicated by the apparent enhanced performance at 2 and 3 months, suggesting that the motor phenotype characterisation of the $Hdh^{Q111}$ mouse would perhaps benefit from additional motor testing (see section 5.6).

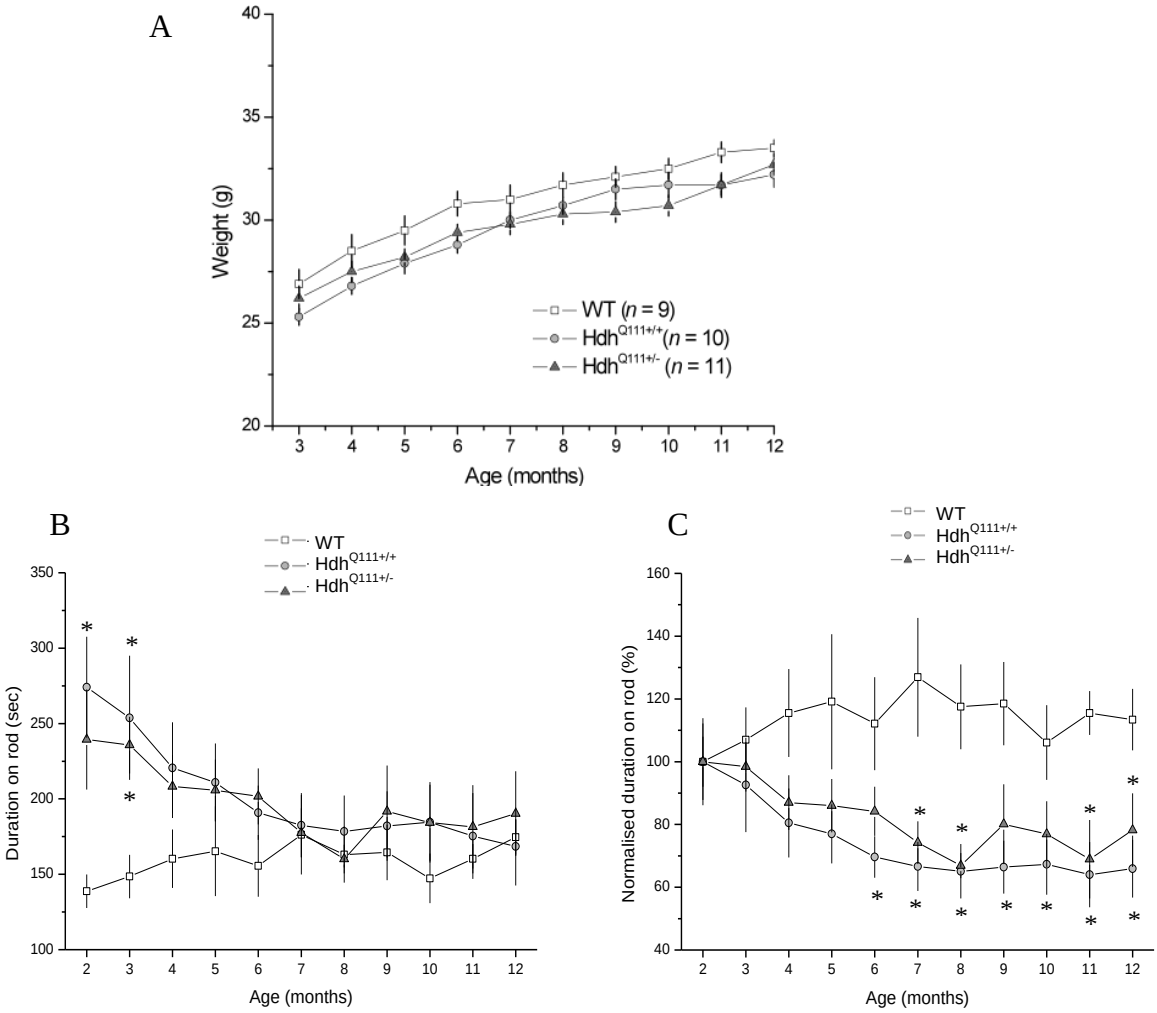



***Figure 5.5: Hdh$^{Q111}$ mice demonstrate a mild motor phenotype on the rotarod.*** *A) WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 11) mice exhibit similar gradual increases in weight over the course of the experiment indicating that the presence of the mutant Huntington protein does not produce any weight discrepancies (P > 0.05). B) Hdh$^{Q111}$ mice show an increased latency to fall from the beam when compared to WT mice at 2 (P < 0.05) and 3 months (P < 0.05). For all of the subsequent months there was no difference between the performances of the three genotypes (all P > 0.05). C) When the data is normalised to baseline performance at 2 months, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice show a consistant performance up to the age of 5 months when compared to their performance at 2 months (all P > 0.05). Hdh$^{Q111+/+}$ mice show an impaired performance by 6 months (P < 0.05), whereas Hdh$^{Q111+/-}$ mice are impaired at 7 months (P < 0.05) when compared to their performance at 2 months. Both Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice are impaired at the rotarod task at 8, 11 and 12 months, when compared to their performance at 2 months, whereas only Hdh$^{Q111+/+}$ mice are impaired at 9 and 10 months (P < 0.05). WT mice show consistant performance on the rotorod as they age (P > 0.05).*

# 5.4: Results: Circular runway

## 5.4.1: $Hdh^{Q111}$ mice show no locomotor deficits at 13 months

The locomotor activity of WT (*n* = 9), $Hdh^{Q111+/+}$ (*n* = 10) and $Hdh^{Q111+/-}$ (*n* = 10) mice was assessed in circular runways (Figure 5.6). A repeated measures ANOVA was performed on the total distances travelled with group (WT *vs*. $Hdh^{Q111+/+}$ *vs*. $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. This showed no overall significant effect of genotype ($f_{(2, 19)} = 0.243$, $P > 0.05$) but a significant effect of day ($f_{(3, 57)} = 2.576$, $P < 0.05$) and a significant day *vs*. genotype interaction ($f_{(6, 57)} = 2.576$, $P < 0.05$). Bonferroni corrected pair-wise comparisons between groups for each day confirmed that the locomotion of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice was similar to WT mice on each of the 4 days (all $P > 0.05$). A mouse was considered to have habituated to the circular runways if the distance travelled by the mice reached a steady but reduced level of activity on each consecutive day. The distance travelled by the WT mice on Day 2 was significantly lower than that travelled on Day 1 ($P < 0.05$), but the distances travelled on Days 3 and 4 were not significantly different from Day 1 (all $P > 0.05$). The WT mice travelled similar distances on Days 2 – 4 ($P > 0.05$). $Hdh^{Q111+/+}$ mice travelled significantly less on Day 2 when compared to Day 1 ($P < 0.05$) but the distances travelled on Days 3 and 4 were not significantly different from Day 1 ($P > 0.05$). The $Hdh^{Q111+/+}$ mice travelled similar distances on Days 2 – 4 ($P > 0.05$). $Hdh^{Q111+/-}$ mice travelled significantly less on Days 2 - 4 ($P < 0.05$) when compared to Day 1. Similar distances were travelled on Days 2 - 4 (all $P > 0.05$). In summary, these data show that, at 13 months, $Hdh^{Q111}$ mice show no locomotor deficits in the circular runways.

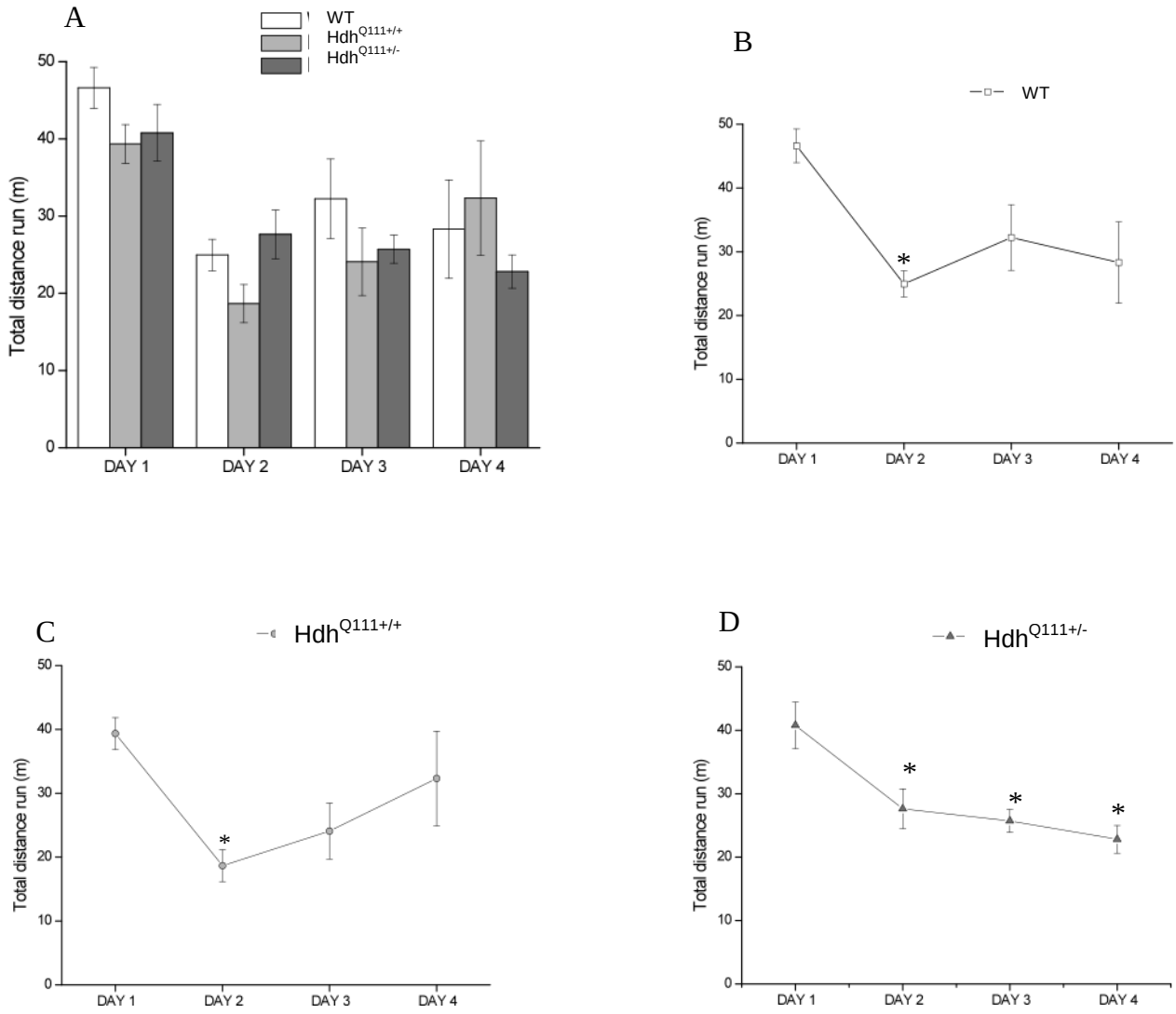



***Figure 5.6: Hdh$^{Q111}$ mice show no locomotor deficits in the circular runways at 13 months.*** *A repeated measures ANOVA indicated no effect of genotype ($f_{(2, 19)} = 0.243$, $P > 0.05$), an effect of day (($f_{(3, 57)} = 2.576$, $P < 0.05$) and a day vs. genotype interaction ($f_{(6, 57)} = 2.576$, $P < 0.05$).*

***(****A) WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) travel similar distances on Days 1-4. The distance travelled by all 3 genotypes differs over the 4 days ($P < 0.05$). (B & C) WT and Hdh$^{Q111+/+}$ mice travel significantly less on Day 2 when compared to Day 1 ($P < 0.05$) but the distances travelled on Days 3 and 4 are not significantly different from Day 1. Similar distances are travelled on Days 2 – 4 (all $P > 0.05$). D) Hdh$^{Q111+/-}$ mice travel significantly less on Days 2 - 4 ($P < 0.05$), when compared to Day 1. Similar distances are travelled on Days 2-4 (all $P > 0.05$).*

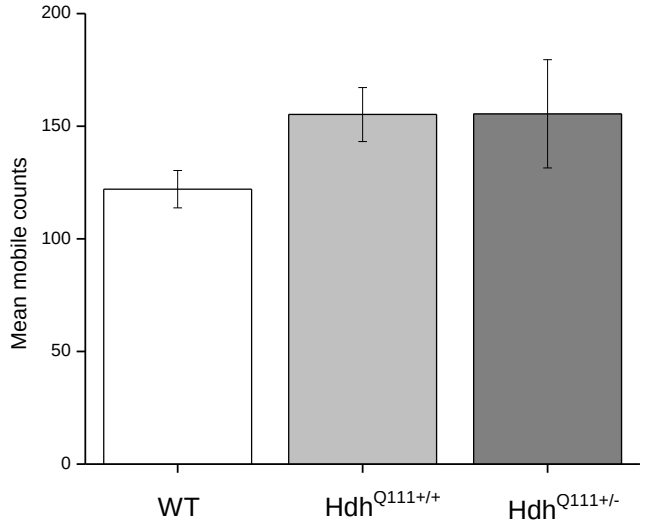
## 5.5: Results: Activity box

The activity box was also used as a measure of locomotor activity in WT (*n* = 9), $Hdh^{Q111+/+}$ (*n* = 10) and $Hdh^{Q111+/-}$ (*n* = 10) mice (13 months). Measurements taken include the mobile counts and mobile time (measurement of how much the mouse is moving around), exploratory rearing (exploration), static counts (freezing behaviour), and active time (measurement of all activity *e.g.* grooming, head dipping and other stereotypical behaviours).

### 5.5.1: Mobile Counts

The total number of mobile counts at the end of the 4 days was assessed. A repeated measures ANOVA was performed with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. Results demonstrated that there was no effect of genotype ($f_{(2, 26)} = 0.759$, $P > 0.05$; Figure 5.7A) or day *vs.* genotype interaction ($f_{(6, 78)} = 1.531$, $P > 0.05$) (Figure 5.8B). There was an effect of day ($f_{(3, 78)} = 7.009$, $P < 0.05$; Figure 5.7B) revealing a general decrease in activity over the four days of testing suggesting inter-session habituation.

A




B

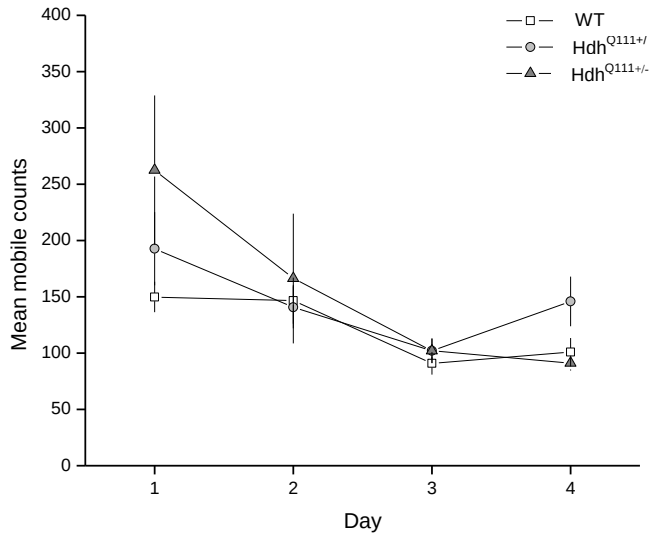



***Figure 5.7: Hdh$^{Q111}$ mice show similar mobile counts in the activity box (13 months)***

*A repeated measures ANOVA indicated no effect of genotype ($f_{(2, 26)} = 0.759$, $P > 0.05$) or day vs. genotype interaction ($f_{(6, 78)} = 1.531$, $P > 0.05$) but an effect of day ($f_{(3, 78)} = 7.009$, $P < 0.05$).*

*A) Histograms showing mean number (± SEM) of mobile counts as measured by beam breaks in WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) mice at the end of a 4 day period.*

*B) Line graph showing the mean number (± SEM) of mobile counts as measured by beam breaks on Days 1 to 4 in WT, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice.*

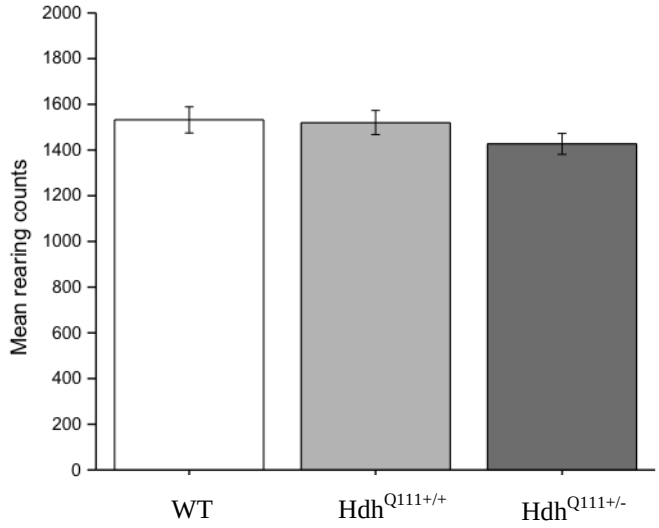
## 5.5.2: Exploratory Rearing

The total number of rearing counts at the end of the 4 days was assessed. A repeated measures ANOVA was performed with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. Results indicated that there was no significant effect of genotype ($f_{(2, 26)} = 1.200$, $P > 0.05$; Figure 5.8A). There was an effect of day ($f_{(3, 78)} = 26.170$, $P < 0.05$; Figure 5.8B) revealing a general decrease in activity over the four days of testing suggesting inter-session habituation. There was no day *vs.* genotype interaction ($f_{(6, 78)} = 0.165$, $P > 0.05$) (Figure 5.8B).

## 5.5.3: Static counts

The total number of static counts at the end of the 4 days was assessed. A repeated measures ANOVA was performed with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. Results indicated that there was a significant effect of genotype ($f_{(2, 26)} = 6.006$, $P < 0.05$; Figure 5.9A). Bonferroni corrected pair-wise comparisons between groups confirmed that the $Hdh^{Q111+/+}$ mice had significantly more static counts than WT and $Hdh^{Q111+/-}$ mice (both $P < 0.05$). WT and $Hdh^{Q111+/-}$ mice showed similar static counts ($P > 0.05$). There was no significant effect of day ($f_{(3, 78)} = 1.574$, $P > 0.05$) or day *vs.* genotype interaction ($f_{(6, 78)} = 1.613$, $P > 0.05$; Figure 5.9B).

A




B

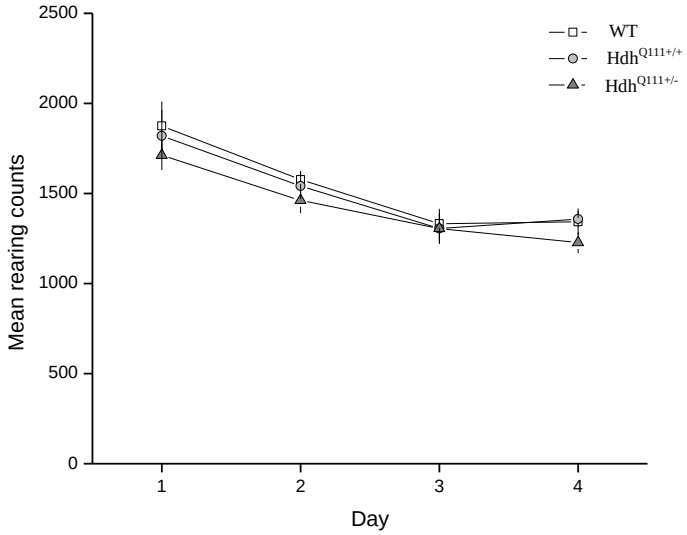


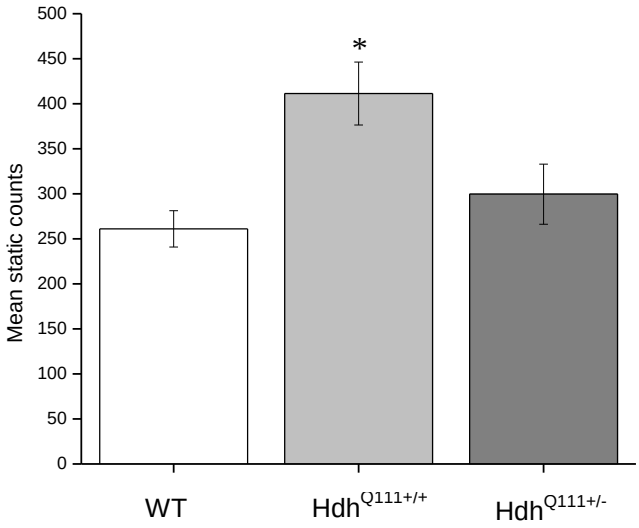

***Figure 5.8: Hdh$^{Q111}$ mice show similar exploratory rearing in the activity box (13 months)***

*A repeated measures ANOVA indicated no effect of genotype ($f_{(2, 26)} = 1.200$, $P > 0.05$), an effect of day ($f_{(3, 78)} = 26.170$, $P < 0.05$) and no day vs. genotype interaction ($f_{(6, 78)} = 0.165$, $P > 0.05$).*

*A) Histograms showing mean number (± SEM) of rearing counts as measured by beam breaks in WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) mice at the end of a 4 day period.*

*B) Line graph showing the mean number (± SEM) of rearing counts as measured by beam breaks on Days 1 to 4 in WT, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice.*

A




B

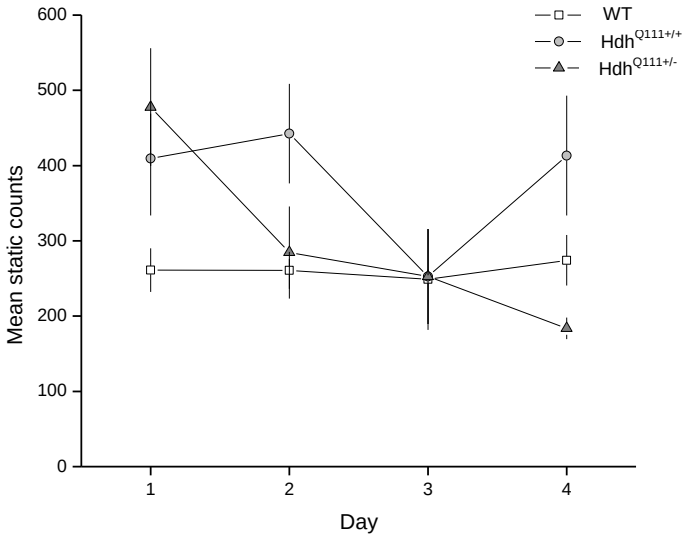



***Figure 5.9: Hdh$^{Q111+/+}$ mice show increased freezing in the activity box (13 months)***

*A repeated measures ANOVA indicated an effect of genotype ($f_{(2, 26)} = 6.006$, $P < 0.05$), no effect of day ($f_{(3, 78)} = 1.574$, $P > 0.05$) and no day vs. genotype interaction ($f_{(6, 78)} = 1.613$, $P > 0.05$).*

*A) Histograms showing mean number (± SEM) of static counts as measured by beam breaks in WT (n = 9), Hdh$^{Q111+/-}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) mice at the end of a 4 day period. Bonferroni pairwise comparisons Hdh$^{Q111+/+}$ mice had significantly more static counts than WT and Hdh$^{Q111+/-}$ mice (both $P < 0.05$), WT and Hdh$^{Q111+/-}$ mice had similar static counts ($P > 0.05$).*

*B) Line graph showing the mean number (± SEM) of static counts as measured by beam breaks on Days 1 to 4 in WT, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice.*

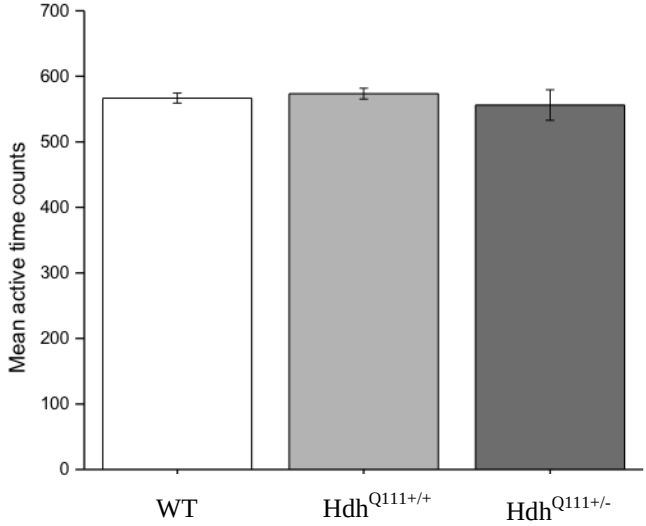
## 5.5.4: Active time

The total number of active time counts at the end of the 4 days was assessed. A repeated measures ANOVA was performed with genotype (WT *vs*. $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. Results indicated that there were no effect of genotype ($f_{(2, 26)} = 0.279$, $P > 0.05$; Figure 5.10A). There was an effect of day ($f_{(3, 78)} = 3.040$, $P < 0.05$) revealing a general decrease in activity over the four days of testing suggesting inter-session habituation. There was no day *vs*. genotype interaction ($f_{(6, 78)} = 0.984$, $P > 0.05$) (Figure 5. 10B).

## 5.5.5: Mobile time

The total number of mobile time counts at the end of the 4 days was assessed. A repeated measures ANOVA was performed with genotype (WT *vs*. $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and day (1 - 4) as the within subjects factor. Results indicated that there was no effect of genotype ($f_{(2, 26)} = 0.219$, $P > 0.05$; Figure 5.11A). There was an effect of day ($f_{(3, 78)} = 18.466$, $P < 0.05$) revealing a general decrease in activity over the four days of testing suggesting inter-session habituation. There was no day *vs*. genotype interaction ($f_{(6, 78)} = 1.037$, $P > 0.05$) (Figure 5.11B).

A




B

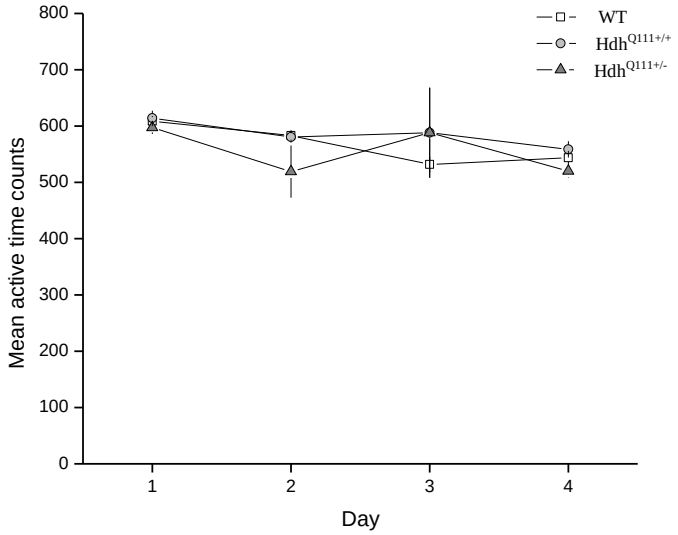


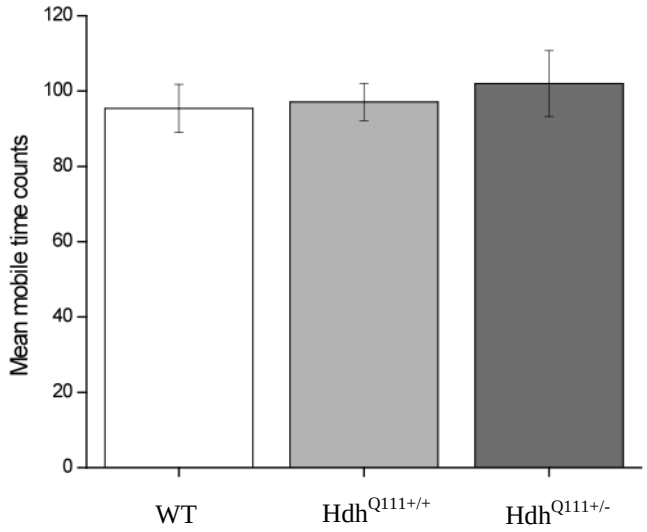

***Figure 5.10: Hdh$^{Q111}$ mice show similar active time counts in the activity box (13 months)***

*A repeated measures ANOVA indicated no effect of genotype ($f_{(2, 26)} = 0.279$, $P > 0.05$), an effect of day ($f_{(3, 78)} = 3.040$, $P < 0.05$) and no day vs. genotype interaction ($f_{(6, 78)} = 0.984$, $P > 0.05$).*

*A) Histograms showing mean number (± SEM) of active time counts as measured by beam breaks in WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) mice at the end of a 4 day period.*

*B) Line graph showing the mean number (± SEM) of active time counts as measured by beam breaks on Days 1 to 4 in WT, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice.*

A




B

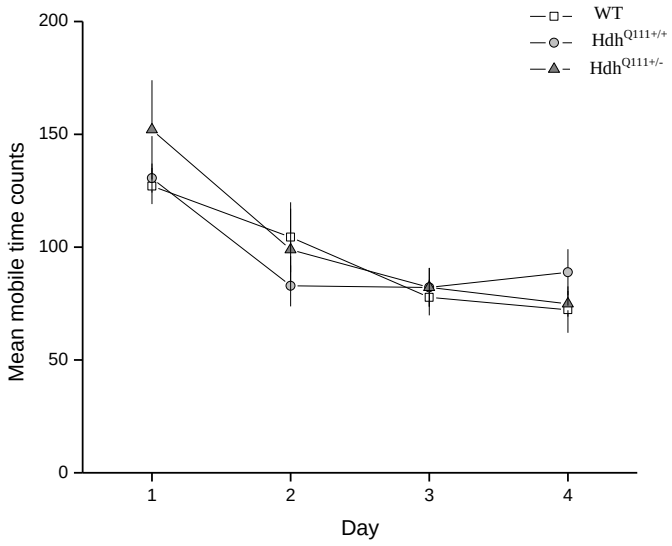



***Figure 5.11: Hdh$^{Q111}$ mice show similar mobile time counts in the activity box (13 months)***

*A repeated measures ANOVA indicated no effect of genotype ($f_{(2, 26)} = 0.219$, $P > 0.05$), an effect of day ($f_{(3, 78)} = 18.466$, $P < 0.05$) and no day vs. genotype interaction ($f_{(6, 78)} = 1.037$, $P > 0.05$).*

*A) Histograms showing mean number (± SEM) of mobile time counts as measured by beam breaks in WT (n = 9), Hdh$^{Q111+/+}$ (n = 10) and Hdh$^{Q111+/-}$ (n = 10) mice at the end of a 4 day period.*

*B) Line graph showing the mean number (± SEM) of mobile time counts as measured by beam breaks on Days 1 to 4 in WT, Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice.*

## 5.6: Discussion

Although they are considered by some to be more relevant genetic models, knock-in mouse models have received relatively little attention in comparison to transgenic models of HD. This is mainly to do with the lack of an obvious motor phenotype in knock-in mouse models at an early age (Shelbourne *et al*., 1999; Wheeler *et al*., 2000; Lin *et al*., 2001). In an attempt to characterise the motor phenotype of the $Hdh^{Q111}$ mouse model of HD, the rotarod was used to assess motor co-ordination, while the circular running tracks and the activity box were used to assess locomotor activity.

The motor co-ordination of the $Hdh^{Q111}$ mouse was assessed using the accelerating rotarod paradigm. Analysis of the raw data suggested that, in agreement with Mennalled and colleagues (2009), $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice demonstrated an increased latency to fall from the rotating rod when compared to WT animals, suggesting an enhanced performance in the $Hdh^{Q111+/+}$ mice at early ages. However, analysis of the normalised data indicated a slight reduction in rotarod performance in $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice from 6 and 7 months respectively, although this was not consistent at all the subsequent ages. However it must be emphasised that as the $Hdh^{Q111}$ mice showed evidence of an enhanced performance at 2 and 3 months, the subsequent reduction in performance on the rotarod could perhaps be indicative of a return to a 'normal' motor phenotype, rather than a display of a motor deficit. Subsequently there is a need for more sensitive tests to be used to more accurately assess the motor phenotype of the $Hdh^{Q111}$ mouse.

The motor co-ordination of rodents can also be tested using a fixed speed protocol, in which subjects are placed on the rotating rod at fixed, increasing speeds. Furthermore,

the fixed speed rotarod has been shown to be more sensitive to striatal dysfunction, a pathological phenotype of HD (Monville *et al*., 2006). In agreement, Hickey *et al.* (2008) demonstrated that while the CAG140 mouse model of HD was not impaired in the accelerating rotarod paradigm, subsequent testing using the fixed speed rotarod protocol revealed deficits when compared to control animals. It would therefore perhaps be beneficial to test the $Hdh^{Q111}$ mice using a fixed speed rotarod protocol in order to determine if a more robust motor deficit can be displayed.

However, even in the more severe transgenic models of HD, *e.g*. the R6/2 mouse, the rotarod does not reveal deficits until after the synaptic, molecular and pathological abnormalities have been detected (Hickey *et al*., 2005; Stack *et al*., 2005). Therefore it would perhaps be of value if the $Hdh^{Q111}$ mouse could be tested using more sensitive tests in an attempt to detect anomalies at an earlier age. Other tests, including the balance beam and foot print analysis, have been used to assess fine motor co-ordination and balance in other mouse models of HD (Carter *et al*., 1999; Hickey *et al*., 2005; Stack *et al*., 2005; Hickey *et al*., 2008) and could perhaps be used to further examine the early motor phenotype of the $Hdh^{Q111}$ mouse model of HD.

The spontaneous locomotor activity was assessed in the circular runways and activity box. The use of circular runways to assess locomotor dysfunction in rodents is advantageous as there are no corners, thereby encouraging spontaneous locomotion in rodents. The lack of abnormalities in the $Hdh^{Q111}$ mouse at 13 months is in agreement with other studies who have demonstrated no differences in exploratory behaviours in the open field test up to the age of 17 months (Wheeler *et al*., 2000; Menalled *et al*.,

2009). However, it is possible that a motor phenotype could perhaps emerge if testing had been extended to include older mice.

Of specific interest, it was demonstrated that the static counts in the activity box differed in the $Hdh^{Q111+/+}$ mice. Analysis of the static counts demonstrated that $Hdh^{Q111+/+}$ mice exhibited an increased freezing response, as indicated by a significant increase in the static counts when compared to WT and $Hdh^{Q111+/-}$ mice. In addition, distinct from both WT and $Hdh^{Q111+/-}$ mice that demonstrated a decrease their static counts over the 4 days, the $Hdh^{Q111+/+}$ mice showed similar "freezing" responses over the course of the experiment. These data indicates that the $Hdh^{Q111+/+}$ mouse may exhibit a slightly anxiogenic phenotype. In order to determine if this is a true phenotype it would be advantageous to assess the anxiogenic phenotype of the $Hdh^{Q111}$ mouse in more depth. Previous studies have identified an anxiogenic phenotype in other rodent models of HD by utilising the open field test and light-dark box (Hickey *et al*., 2005; Mennalled *et al*., 2009; Pang *et al*., 2009; Pouladi *et al*., 2009). In particular, a study utilising a novelty suppressed feeding task has recently shown that an anxiogenic phenotype has been observed in the $Hdh^{Q111}$ mouse prior to the emergence of an overt motor phenotype (Orvoen *et al*., 2012). Collectively these studies suggest that anxiety may be an early feature of HD (Hickey *et al*., 2005; Mennalled *et al*., 2009; Pang *et al*., 2009; Pouladi *et al*., 2009; Orvoen *et al*., 2012), a psychiatric feature which is also present in human patients of HD (Sturrock and Leavitt, 2010). As a possible anxiogenic phenotype is suggested by the increased freeze response of the $Hdh^{Q111+/+}$ mice in the activity box, it would perhaps be valuable to assess the anxiogenic phenotype by utilising the light-dark box and novelty suppressed feeding task, to examine this phenotype more thoroughly.

# 6: Experiment 2: Characterisation of the cognitive phenotype of the $Hdh^{Q111}$ mouse

## 6.1: Introduction: HD and memory

### 6.1.1: Cognitive deficits in human patients of HD

A number of studies have attempted to characterise the early subtle cognitive changes in human patients with HD (Hahn-Barma *et al*., 1998; Lawrence *et al*., 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007). All subjects in these studies were prodromal and free of the psychiatric (*i.e.* depression and anxiety) and motor deficits associated with HD, including chorea and tremors. In these studies the memory of carriers and non-carriers of the *Huntingtin* gene was assessed using either the Wechsler memory scale (WMS) (Hahn-Barma *et al*., 1998; Lawrence *et al*., 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007), the California verbal learning test (CVLT; Hahn-Barma *et al*., 1999; Verny *et al*., 2007), or the Cambridge Neuropsychological Test Automated Battery (CANTAB; Lawrence *et al*., 1998). The WMS is designed to test multiple features of human memory, which are categorised into auditory memory, visual memory, visual working memory, immediate memory, and delayed memory whereas CVLT tests allows the quantification and qualification of short and long term recognition and recall (Hahn-Barma *et al*., 1999). The CNTB test examines various aspects of cognitive function, including working memory and executive function, visual memory, attention and reaction time, semantic memory, decision making and episodic memory (Lawrence *et al*., 1998). In addition, the subjects were tested with the Wechsler Adult Intelligence Scale-Revised (WAIS-R), a psychometric test used to assess basic linguistic function

(Hahn-Barma *et al*., 1999; Lawrence *et al*., 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007). Results indicated that there were significant differences in the cognitive performance of non-carriers and carriers of the *Huntingtin* gene, including impairments in logical memory, short/long term recall and recognition, episodic memory, attention and verbal fluency (Hahn-Barma *et al*., 1998; Lawrence *et al.*, 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007). The results of these studies therefore suggest that cognitive changes, without overt motor, or psychiatric disturbances, represent the first stages of HD (Hahn-Barma *et al*., 1998; Lawrence *et al.*, 1998; Kirkwood *et al*., 2000; Verny *et al*., 2007). It is these cognitive deficits in short/ long term recognition and episodic memory that I aim to test in the $Hdh^{Q111}$ mouse in order to demonstrate that the $Hdh^{Q111}$ mouse is an accurate cognitive model of HD (see section 6.2).

## 6.1.2: Cognitive deficits in animal models of HD

Several studies have assessed the cognitive performance of animal models of HD in an attempt to detect the early cognitive deficits associated with the human disorder (Lione *et al*, 1999; Van Raamsdonk *et al*., 2005; Pang *et al*., 2006; Nithianantharajah *et al*., 2008; Simmons *et al*., 2009). For more details on the genetics of the mouse models described in this section, please see section 1.2.

Many studies have demonstrated a role of the hippocampus in spatial memory. The MWM has been used to test the spatial learning in the R6/2 mouse (testing from 3 weeks of age, over 19 days) (Lione *et al*., 1999). In this study the mouse was trained to find a visible platform within a circular pool. Following training, the platform was submerged and the mouse was trained to swim to the hidden platform. The platform was subsequently removed and the swimming path of the mouse was examined to

assess memory for the original location of the platform. During a reversal test the platform was moved to another location in the pool, and the ability of the mouse to learn the new position of the platform was assessed. Results indicated that R6/2 mice showed similar performance in the visible platform version of the MWM task (Lione *et al*., 1999). However, when the platform was removed, the R6/2 mouse spent significantly less time in the original location of the platform when compared to control (Lione *et al*., 1999). Furthermore, R6/2 mice took less direct routes and took longer to locate the new platform position during the reversal learning phase and spent less time in the location of the platform when it was removed for the reversal test. Although the visible platform version of the MWM is a test of spatial memory, it is not dependent on the hippocampus. On the other hand, the impairments of the R6/2 mice in the hippocampus-dependent hidden platform and reversal test versions of the MWM emphasise the role of the hippocampus in the early cognitive deficits of HD (Rosas *et al*., 2003). Considering the role of the hippocampus in episodic memory (see section 3.1.2), it is possible that, using appropriate behavioural testing, episodic memory deficits could be displayed in the $Hdh^{Q111}$ model of HD.

The T-maze has been used to test spatial learning in the R6/2 (5 – 6.5 weeks; Lione *et al*., 1999), R6/1 (14 weeks; Pang *et al*., 2006) and YAC128 (8.5 months; Van Raamsdonk *et al*., 2005) mouse models of HD. During 'forced' alteration training, one of the T-Maze arms was blocked (*e.g*. the right arm), forcing the mouse to enter the other arm to receive a food reward (*e.g*. the left arm; Lione *et al*., 1999; Pang *et al*., 2006). During the first trial the mouse had a 'free choice' and a reward was placed at the end of both arms. On each subsequent trial the mouse was considered to have made a correct choice if it entered the arm that it had not previously visited. The process was

repeated until the mouse had successfully completed the required number of trials with the number of correct trials/ errors being used as an indication of memory. In the study by Van Raamsdonk *et al.* (2005), a swimming T-Maze was used and the mouse was trained to locate a floating platform in alternate arms of the T-Maze. The results of all 3 of these studies indicated that the mouse models of HD made more errors when compared to control animals in the T-maze (Lione *et al*., 1999; Pang *et al*., 2006; Van Raamsdonk *et al*., 2005). Similar deficits were found in studies in which the short-term spatial memory of the R6/1 mouse was tested using the Y-Maze (Pang *et al*., 2006; Nithianantharajah *et al*., 2008). Similar to the T-Maze, in the Y-Maze task the mice were tested for their ability to identify the arm they had not previously entered (*i.e*. the novel arm), although in this test the length of time spent in the novel arm was used as an indication of memory. Results indicated that while at 12 - 14 weeks, control animals spent an increased amount of time in the novel arm, the R6/1 mouse showed no preference, spending an equal amount of time in all 3 arms (Pang *et al*., 2006; Nithianantharajah *et al*., 2008). These studies therefore provide evidence of short-term hippocampal-dependent memory deficits in mouse models of HD (Lione *et al*., 1999; Pang *et al*., 2006; Van Raamsdonk *et al*., 2005; Nithianantharajah *et al*., 2008). However, as the T-maze required training in which forced alteration behaviour was rewarded, it can be argued that it is not necessarily the spontaneous behaviour of the animal that is being assessed. As episodic memory is formed by a single exposure to an event (section 3.1.1 and 3.1.2.), the development of a hippocampal-dependent test that assesses spontaneous cognitive behaviour, without the need for training and/ or a reward would therefore benefit the assessment of episodic memory in the $Hdh^{Q111}$ mouse.

Interestingly, the cognitive processes of the R6/1 (12 and 14 weeks; Nithianantharajah *et al*., 2008) and CAG140 (16 weeks; Simmons *et al*., 2009) mouse models of HD have been tested using the novel object recognition task (see sections 3.4.1 and 6.1.2). The novel object recognition task does not require any training and assesses memory by utilising the spontaneous novelty seeking behaviour of rodents and therefore does not rely on positive reinforcement, such as rewards, that could alter behaviour. In the study by Nithianantharajah and colleagues (2008), the short-term memory of the mice was tested using the novel object recognition task (section 3.4.1, Figure 3.1A). Results indicated that, at 12 and 14 weeks, both the R6/1 and control animals show similar preference for the novel object. However, R6/1 mice show impairments in location memory (see section 3.4.3, Figure 3.1E; Nithianantharajah *et al*., 2008). Previous studies have suggested that location memory is heavily dependent on the hippocampus (Ennaceur *et al*., 1997; Mumby *et al*., 2002 – see section 3.4.3), while the novel object task is not hippocampus-dependent (Bussey *et al*., 2000; Mumby *et al*., 2002; Langston and Wood, 2010 – see section 3.4.1), perhaps providing a reason for the differences in cognitive impairments in this study and again implicating the hippocampus in the early cognitive deficits of HD (Nithianantharajah *et al*., 2008).

Previous studies have also indicated that the hippocampus could be involved in long-term novel object recognition tasks (Clark *et al*., 2000; Broadbent *et al*., 2004 – see section 3.4.1). In the study by Simmons and colleagues (2009) $Hdh^{Q140}$ mice were tested for 60 minutes over 4 consecutive days in an open-field environment. On Day 1 the open field did not contain any objects. On testing Days 2 and 3 two identical objects were added to the arena. On Day 4 one of the objects was replaced with a novel object. Although wild type mice showed increased exploration at the novel object, $Hdh^{Q140}$

mice were impaired in their ability to identify the novel object when compared to wild type mice following a 24 hour delay. These data indicates that a long-term recognition memory deficit is present in the $Hdh^{Q140}$ mouse model of HD at 16 weeks.

In summary these studies indicate that the cognitive decline occurs in human patients and mouse models of HD prior to the onset of motor abnormalities. Cognition deficits in human patients of HD include impairments in short/long term recognition memory and episodic memory. Furthermore, these cognitive deficits are also seen in various rodent models of HD, specifically in tests that have been shown to involve the hippocampus. Collectively, these studies suggest that cognitive decline could act as an early marker of HD in mouse models, mirroring the progression of the clinical symptoms seen in humans. In this thesis the cognitive performance of the $Hdh^{Q111}$ mouse model of HD will be assessed by utilising the intrinsic novelty-seeking behaviour characteristic of rodents. As no training and/ or positive reinforcers are required, it can be argued that tests assessing the spontaneous behaviour of mice are therefore more indicative of natural behaviour of the mouse. Similar to the studies discussed previously (Nithianantharajah *et al*., 2008; Simmons *et al*., 2009) the short- and long-term memory of $Hdh^{Q111}$ mice will be assessed using the novel object recognition task in order to model the recognition memory deficits seen in HD patients. Furthermore, in an attempt to replicate the episodic memory deficits associated with HD, the novel object recognition test will be extended to test the 'what', 'where' and 'which' of episodic-like memory (Eacott and Norman, 2004; Langston and Wood, 2010; see section 3.4.2).

# 6.2: Methods

## 6.2.1: Assessment of episodic memory: long protocol

In order to assess episodic memory, it is necessary to demonstrate that $Hdh^{Q111}$ mice are also capable of demonstrating memory for the individual components of episodic memory. The following section will present the methods for the novel-object, object-place, object-context and object-place-context (episodic memory) tasks.

### 6.2.1.1: Subjects

WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (male and female, 2 and 13 months) were used as subjects. Note, in the cognition experiments in the following chapters, the age stated at the beginning of each section represents the age at which the cognition testing commenced. All mice were housed in groups of 2-6 and kept on a 12 hour light/ 12 hour dark cycle (dawn at 5am, dusk at 5pm). All mice had unrestricted access to water and food throughout the experiment.

### 6.2.1.2: Apparatus

All testing was carried out in a rectangular arena (width = 24 cm; length = 28 cm; depth = 20 cm) (Figure 6.1). The walls of the arena were interchangeable in order to create two different contexts. Context 1 consisted of a floor and wipeable white walls. Context 2 consisted of a white plastic floor insert with holes across its surface. The original floor of context 1 remained exposed through these holes to emphasise that it was the same arena in the same location and that only local features had been altered. The walls of context 2 consisted of 4 cardboard inserts, covered with a wipeable green tile-effect wall paper. Reusuable Velcro strips were placed in the same location on each of the

floor inserts on which the objects were always presented. These locations were centrally located within the north-west and north-east quadrants of the box. At the north-west and north-east corners of the box, large 3D visual cues were hung from the wall. These cues were maintained in a constant position in the testing room relative to the testing box. The wall and floor inserts were always placed in the same location and orientation to control for any local cues present on the testing apparatus. The configuration of the box as either context 1 or context 2 was counterbalanced across the genotypes for each task. In addition, each context was wiped down with lemon antibacterial wipes (Tesco) in between each time a mouse entered the testing box.

For each task, the exploration times of the mice at each object were recorded. Exploration was defined as the mouse being within approximately 2 cm of the object, directing its nose at the object and being actively involved in exploration *e.g.* sniffing or whiskers twitching. If the mouse was sitting on or next to the object with no signs of exploration, these time periods were not included. Objects for exploration were collected from a variety of sources but had to fulfil the criteria of being easily cleaned, made from nonporous materials and either heavy enough that the mice could not push them over or having a suitable base where a Velcro strip could be attached. Objects were always presented in the same locations and orientations in the testing box (see Figure 6.1).

#### 6.2.1.3: Behavioural testing

All tests were carried out in the light phase 4 days a week. The mice were tested on the novel-object, object-place, object-context, and then object-place-context task.

#### 6.2.1.4: Habituation

Prior to experimentation, the mice were removed from their home cage and handled in order to ensure that the mice were used to the experimenter. Initial habituation sessions were carried out before behavioural testing commenced to familiarise the mice with the arena, the two different contextual configurations of the testing arena and the locations in which these objects would be placed. Each day, the mice were brought into the testing room in their home cage (2 – 6 mice), which was placed on a trolley near the test arena. On Day 1 the mice were placed within the arena, which was arranged in 1 of the 2 contexts, for 15 minutes in their cage groups. The animals were then removed and placed back into the home cage, while the context configuration was changed. The animals were then replaced back into the arena in their cage groups for a further 15 minutes before being returned to their home cage. On Day 2, the mice were placed in one of the two contexts individually for 5 minutes, placed in a holding chamber, an opaque bucket containing sawdust, while the context was changed and then placed back in the arena for the second context for an additional 5 minutes before being returned to their home cage. Care was taken to ensure that the mouse was placed within the arena at the same position, facing the south wall, as would happen during testing. No objects were present in the testing arena on Days 1 and 2. On Day 3 and 4, mice were placed in one of the two contexts and two objects were placed within the arena in the north-west and north-east corners; alternate contexts and different objects were used on each day. Days 3 and 4 ensured that the mice were familiar with the locations in which the objects would appear. Each object was presented only once during habituation and did not appear at any later stage of testing.

#### 6.2.1.5: Object recognition task

Two days after the end of habituation, mice were tested on the novel object recognition test to ensure that the animals in both groups were able to discriminate between objects (Figure 6.1A).

On each trial, the mouse to be tested was removed from its home cage and placed in the holding box next to the test arena. In the sample phase, the mouse was timed exploring the two identical objects in the testing box for 3 minutes after which the mouse was removed from the arena and placed in the holding box. After a short delay (~2 minutes), the mouse was presented with one object that was a third copy of the objects seen in the sample phase while the other object was completely novel. This test phase was carried out using the same procedures as the sample phase. After each test phase, the animal was returned to its home cage. Each mouse received four trials of the novel object recognition task with an inter-trial interval of 24 hours. Two trials took place in context 1 and two trials took place in context 2. For two trials, one in each context, the novel object occurred on the left and for the other two it occurred on the right.

#### 6.2.1.6: Object-place task

The procedure for the mice in the object-place task was similar to that used in the object recognition task, except that the objects in the sample and test phases were manipulated differently (Figure 6.1B). During the sample phase, two different objects were present in the test arena. During the test phase, which occurred in the same context as the sample phase, a further two copies of one of the two objects presented in the sample phase was presented. Thus, during the test phase, one of the objects was presented in the same place as it had previously been in the sample phase, whereas the other was in a

place that had previously been occupied by a different object. In this situation, both the objects and the places were familiar but for one object the object-place configuration was novel. Each mouse received four trials on the object-place task with an inter-trial interval of 24 hours. Two trials took place in context 1 and two trials took place in context 2. For two trials, one in each context, the novel object-place configuration occurred on the left and for the other two it occurred on the right.

#### 6.2.1.7: Object-context task

The procedure for the mice in the object-context task was similar to that used in the previous tasks but there were two sample phases, one in each context, followed by a single test phase (Figure 6.1C). Between each sample phase the mouse was removed and placed in the holding box while the test arena was cleaned and reconfigured. During the first sample phase (Sample 1), two identical copies of an object were placed within the test arena, which was configured as either context 1 or 2. For the second sample phase (Sample 2), the testing arena was reconfigured as the opposite context and two identical copies of another object were presented. For the test phase, the test arena was reconfigured as either the context from Sample 1 or remained in the same context as Sample 2. One copy of the object from Sample 1 and one copy of the object from Sample 2 were present. Thus, the objects, their absolute locations, and the contexts were all familiar by the test phase, but for one object the object-context configuration was novel. Each mouse received four trials on the object-context task. The trials were arranged in such a way that counterbalanced for the effects of relative recency on memory for the objects or the contexts. For two trials, one in each context, the novel object-context configuration occurred on the left and for the other two it occurred on the right.

#### 6.2.1.8: Object-place-context task

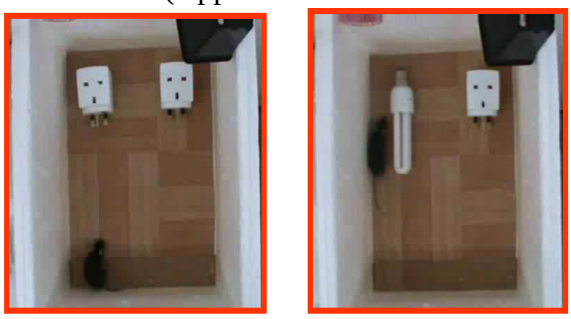
The procedure for the mice in the object-place-context task was similar to that used in the object-context task. During the first sample phase (Sample 1), the test arena was configured as either context 1 or context 2, and two different objects were placed in the test arena (*e.g*. object A on the left and object B on the right) (Figure 6.1D). During Sample 2, the box was configured as the context not used in Sample 1, and identical copies of the same two objects used during Sample 1 were present, but their locations were swapped relative to Sample 1 (*e.g.* object B on the left and object A on the right). For the test phase, the test arena was either reconfigured as the context used in Sample 1 or remained in the same context as Sample 2. During the test phase, two identical copies of one of the objects from the sample phases were present. Thus, one of the object copies was in an object-place-context configuration in which it had not been previously experienced (novel). Each mouse received four trials on the object-place-context task. The trials were arranged in such a way that counterbalanced for the effects of relative recency on memory for the objects or the contexts. Again, for two trials, one in each context, the novel object-place-context configuration occurred on the left and for the other two it occurred on the right.

#### 6.2.1.9: Data collection and analysis

The mice were monitored by an overhead video camera (Sony HandyCam) and viewed by the experimenter on a television monitor (LG). The time spent exploring was recorded manually, using key press-activated timers to time exploration at the left and right objects before being entered into a Microsoft Excel spreadsheet, in which further analysis was performed. SPSS software was used for all statistical analysis. Note, in all the following results chapters, the statistical test used is defined in the appropriate section. The exploration times at each object was noted in both the sample and test

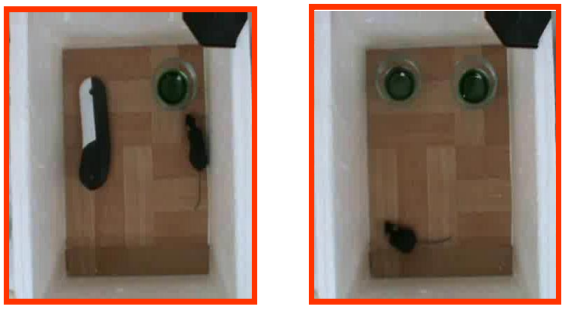
phases in order to provide a comparison of exploration between genotypes. In the object-context and object-place-context tasks, the exploration times from each sample phase were combined and used to calculate a mean exploration. In addition, the raw times spent at each object in the test phase were converted into a discrimination index for each mouse using the formula (time at novel – time at familiar) / (time at novel + time at familiar) where novel refers to the novel object and familiar refers to the other object. A value of zero indicates no preference, whereas as a positive value indicates preferential exploration of the novel configuration. For each mouse, a discrimination score for each task was obtained by calculating the mean of the discrimination indices for the four trials on that task

A: Novel Object (Apparatus dimensions w=24 cm; l=28 cm; d=20 cm)



Sample Test

B: Object-place



Sample Test

C: Object-context

Sample 1 Sample 2 Test

D: Object-place -context

Sample 1 Sample 2 Test

***Figure 6.1**: **Representation of the apparatus used to show episodic memory.** (A), object-place (B), object-context (C) and object-place-context task (D). Context 1 consisted of a wooden floor and wipeable white walls. Context 2 consisted of a white plastic floor insert with holes across its surface. Objects were presented in the same locations for each trial. Mice were placed into the testing apparatus from the south side, facing the south wall for each sample and test phase.*

## 6.2.2: Assessment of episodic memory: short protocol

As it was important to identify cognitive deficits at the earliest possible time point, it was necessary to assess cognition in a precise time-specific manner. Therefore, in addition to the long protocol, a shorter protocol was also used to assess episodic memory. The general protocol was similar to that stated previously (section 6.2.1) but differed in the way that instead of taking 5 weeks to complete, the mice had completed all 4 tasks in 3 days (Figure 6.1).

Mice were habituated to the test arena in the same way as stated previously (section 6.2.1.4), although habituation was over the course of 1 day, instead 4. The mice were subsequently tested on the spontaneous recognition memory tasks on Days 2 and 3. The testing process was identical to that of the long protocol but instead of each mouse carrying out each task 4 times and an average calculated, each task was only carried out by each mouse once. On Day 2 the mice were tested on the novel-object recognition task in the morning and the object-context task in the afternoon. On Day 3 the mice were tested on the object-place task in the morning and the object-place-context task in the afternoon. Discrimination indices from each task were calculated as stated previously (section 6.2.1.9).

## 6.2.3: 24 hour novel object recognition task

The hippocampus has been linked to the early cognitive deficits in HD (Rosas *et al*., 2003). Studies have demonstrated that, at long delays, novel object recognition can be dependent on the hippocampus (section 3.4.1). In order to provide another cognitive test that could assess the integrity of the hippocampus in HD, the 24 hour novel object recognition test was used to assess long-term memory in $Hdh^{Q111}$ mice.

#### 6.2.3.1: Subjects

WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (1 and 2 months) were used as subjects. Mice were kept in the same conditions as stated previously (section 6.2.1.1)

#### 6.2.3.2: Apparatus

Due to the fact that some of the mice participating in the 24 hour novel object recognition task were also tested using the previous recognition tasks (section 6.2.1 and 6.2.2), it was necessary to produce a different arena in order to avoid interference between tests. Therefore the 24 hour novel object task was carried out in a circular arena (width = 37 cm; depth = 19 cm; Figure 6.2). The walls of the arena were covered in a wipeable brown laminate effect paper. Re-usuable Velcro strips were placed in the same location on each of the floor inserts on which the objects were always presented. These locations were located centrally within the north-west and north-east quadrants of the box (Figure 6.2).

#### 6.2.3.3: Habituation

Initial habituation sessions were carried out before behavioural testing commenced to familiarise the mice with the test arena. Each day, the mice were brought into the testing room in their home cage (2 - 6 mice), which was placed on a trolley near the test arena. On Day 1 the mice were removed from their home cage and handled in order to ensure that the mice were used to the experimenter. On the morning of Day 2, the mice were placed within the arena in their home cage groups for 15 minutes. The animals were then removed and placed back into their home cage. In the afternoon of Day 2 the mice were placed into the empty arena individually for 10 minutes each before being removed and placed back in their home cage.

### 6.2.3.4: 24 hour novel object recognition task

After habituation the mice were tested in the 24 novel object recognition task. The 24 hour novel object task differed from that stated previously (section 6.2.1.5) in as far as each mouse participated in 2 sample phases over 2 days (Days 3 and 4; Figure 6.2). The mouse was timed exploring the two identical objects in the test arena for 10 minutes. On Day 5 (Test phase), 24 hours after sample 2, one object was replaced with a novel object and again the time spent exploring each object was recorded for 10 minutes.

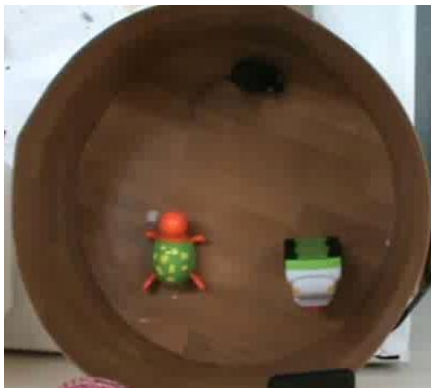
### 6.2.3.5: Data collection

Data was collected as stated in section 6.2.1.9. A record of the exploration at each object was made half way through the experiment (5 minutes) and at the end (10 minutes) of the test phase. The discrimination index was calculated as indicated in section 6.2.1.9.

A: Sample 1

B: Sample 2

C: Test phase



(Apparatus dimensions w=37 cm; d=19 cm)

***Figure 6.2: Representation of the apparatus used in the 24 hour novel object recognition task****. Following habituation on Day 1, the sample phases (10 minutes) on Days 2 and 3 consist of 2 identical objects (A and B). In the test phase on Day 4 (C), one of the objects is replaced with a novel object. Exploration at each of the objects was recorded, taking note of the exploration times at both 5 and 10 minutes.*

## 6.3: Results: Assessment of episodic memory (2 months)

Episodic memory requires the integration of the 'what', 'where' and 'when' components of memory (Tulving, 1972; section 3.3). Previous studies have demonstrated that rats are capable of identifying the 'what', 'where' and 'which' of episodic-like memory (Eacott and Norman, 2004; Langston and Wood, 2010; section 3.4.2). However, this study is the first time this test has been utilised in mice. In order to assess episodic memory in $Hdh^{Q111}$ mice, it is necessary to demonstrate that they are also capable of demonstrating memory for the individual components of episodic memory. $Hdh^{Q111}$ mice were tested in the novel-object, object-place, object-context and object-place-context (episodic memory) tasks. The discrimination indices of WT ($n$ = 24), $Hdh^{Q111+/+}$ ($n$ = 16) and $Hdh^{Q111+/-}$ ($n$ = 24) mice in each of these tasks were evaluated (2 months). A repeated measures ANOVA was performed on discrimination scores with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and task (novel-object *vs*. object-place *vs*. object-context *vs.* object-place-context) as the within subjects factor. Results showed a significant effect of genotype ($f_{(2, 59)} = 41.097$, $P < 0.05$), task ($f_{(3, 59)} = 162.329$, $P < 0.05$) and a significant task *vs.* genotype interaction ($f_{(6, 59)} = 35.381$, $P < 0.05$) (2 months). The next section will report the performance of $Hdh^{Q111+/+}$, $Hdh^{Q111+/-}$ and WT mice in each individual task at 2 months.

### 6.3.1: Object recognition task

WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (2 months) were initially tested in an object recognition test. Bonferroni corrected pair-wise comparisons between groups confirmed that the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice did not differ from WT mice in the object recognition task ($P > 0.05$; Figure 6.3A). In addition, one-sample *t*-tests showed that all three genotypes explored the novel object significantly more than

expected by chance. These data show that the presence of the mutant *Huntingtin* gene does not impair the ability of the mice to recognise a novel object/remember a familiar object at 2 months.

The exploration times in the object recognition task were assessed. One way ANOVAs showed there were no significant differences in the exploration patterns of the mice in the sample ($f_{(2, 61)} = 0.064$, $P > 0.05$) and test phase ($f_{(2, 61)} = 0.998$, $P > 0.05$; 2 months) indicating that the *Huntingtin* mutation does not affect exploration (Figure 6.4A).

### 6.3.2: Object-place and object-context

The mice were then tested in the object-place and object-context tasks. Bonferroni corrected pair-wise comparisons between groups for each task confirmed that $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice did not differ from WT mice in the object-place or object-context tasks ($P > 0.05$) at 2 months (Figure 6.3B and 6.3C). In addition, the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice was similar in both of these tasks ($P > 0.05$). Finally, one-sample *t*-tests showed that all three genotypes explored the novel configuration significantly more than expected by chance in both the object-place and object-context tasks ($P < 0.05$). These data show that the presence of the mutant *Huntingtin* gene does not impair the ability of the mice to recognise a novel/remember a familiar place or context configuration at 2 months.

The exploration times in the associative memory tasks were assessed. One way ANOVAs showed no significant differences in the exploration patterns of the mice in the sample ($f_{(2, 60)} = 0.257$, $P > 0.05$) and test phase ($f_{(2, 60)} = 1.001$, $P > 0.05$) of the object-place task (2 months; Figure 6.4B). In addition, one way ANOVAs demonstrated

no significant differences in the exploration patterns of the mice in the sample ($f_{(2, 61)}$ = 1.517, P > 0.05) and test phase ($f_{(2, 61)}$ = 1.698, P > 0.05) of the object-context task (2 months) again demonstrating that the *Huntingtin* mutation does not affect exploration (Figure 6.4C).

### 6.3.3: Object-place-context task: Episodic memory

The mice were then tested for episodic memory in the object-place-context task. Bonferroni corrected pair-wise comparisons between groups for each task confirmed that the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice significantly differed from WT mice in the object-place-context task at 2 months (both P < 0.05; Figure 6.3D). Bonferroni corrected pair-wise comparisons between each pair of task for the WT mice indicated no significant differences between tasks (all P > 0.05). In contrast, the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice performed significantly worse in the object-place-context task when compared to the other tasks (both P < 0.05), with no difference between the object-place and object-context tasks (P > 0.05). Finally, one-sample *t*-tests showed that the WT mice (P < 0.05), but not $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (P > 0.05), explored the novel configuration significantly more than expected by chance in the object-place-context task. These data show that the presence of the mutant *Huntingtin* gene impairs the ability of the mice to recognise a novel/remember a familiar object-place-context configuration. These data therefore implies that an episodic memory deficit exists in the $Hdh^{Q111}$ mouse at 2 months.

The exploration times in the object-place-context task were assessed. One way ANOVAs showed no significant difference in the exploration patterns of the three genotypes in the sample ($f_{(2, 61)}$ = 0.031, P > 0.05 and test phase ($f_{(2, 61)}$ = 0.177, P >

0.05) of the object-place-context task (2 months) (Figure 6.4D). The lack of exploration differences between genotypes is particularly important in this task as it indicates that the selective impairment in the object-place-context task is not due to secondary changes in exploratory behavior in the $Hdh^{Q111}$ mice. Interestingly, although assessment of the motor phenotype of $Hdh^{Q111}$ mice revealed enhanced performance on the rotarod at 2 and 3 months (section 5.3.2), the lack of differences in the exploration patterns of the $Hdh^{Q111}$ using this paradigm again suggests that further motor testing in the $Hdh^{Q111}$ mouse is required to elucidate a more accurate motor phenotype.

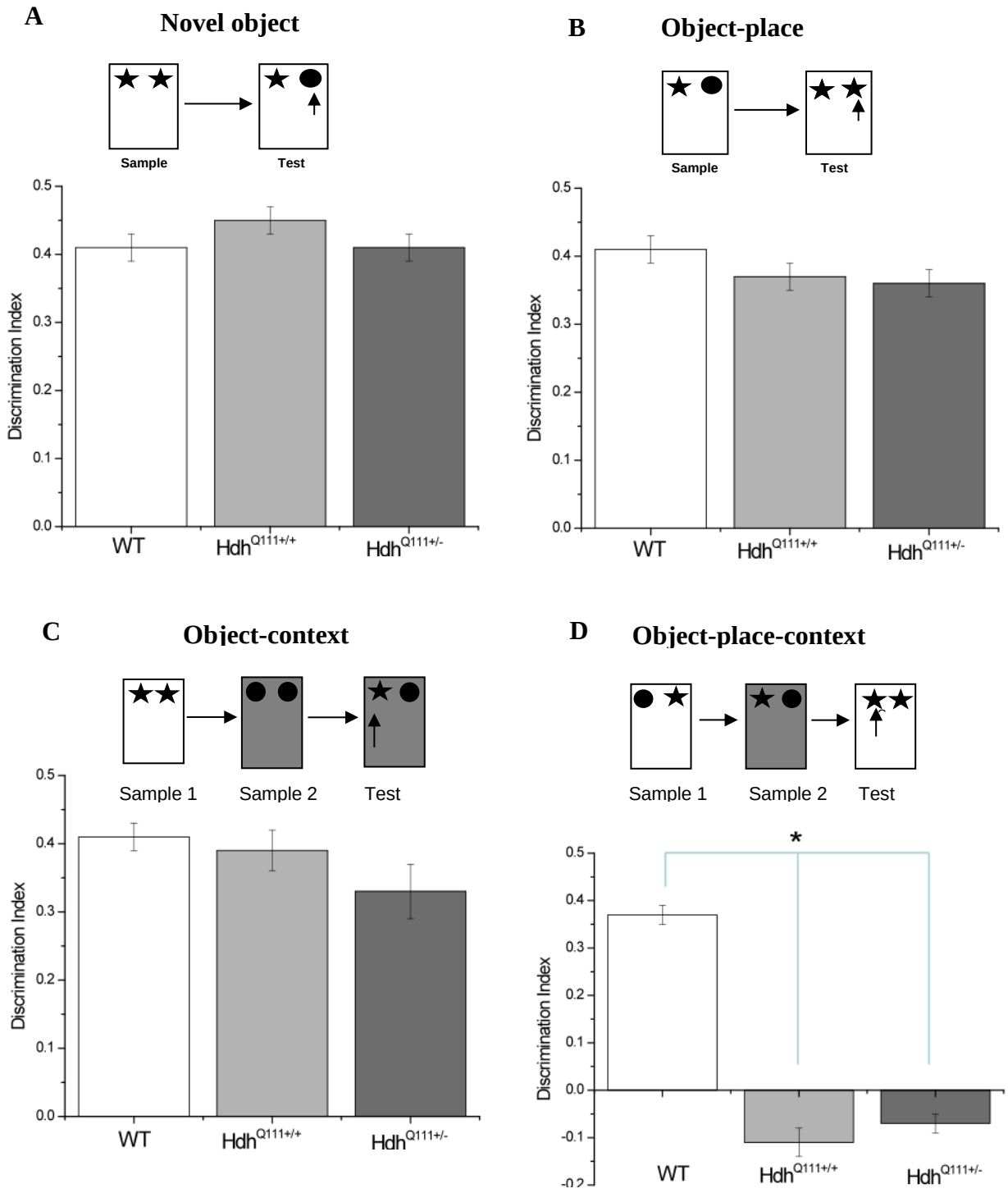



***Figure 6.3: Hdh$^{Q111}$ mice show impairments in episodic memory at 2 months.***

*For each panel: Top shows schematic representation of the task. Bottom shows discrimination ratios of WT (n = 24), Hdh$^{Q111+/+}$ (n = 16) and Hdh$^{Q111+/-}$ (n = 24) mice (2 months). All genotypes show similar ability to identify the novel configuration in the (A) novel object, (B) object-place (C) and object-context tasks (all P > 0.05). (D) Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice are significantly impaired in their ability to identify the novel configuration in the object-place-context task when compared to WT mice (P < 0.05).*

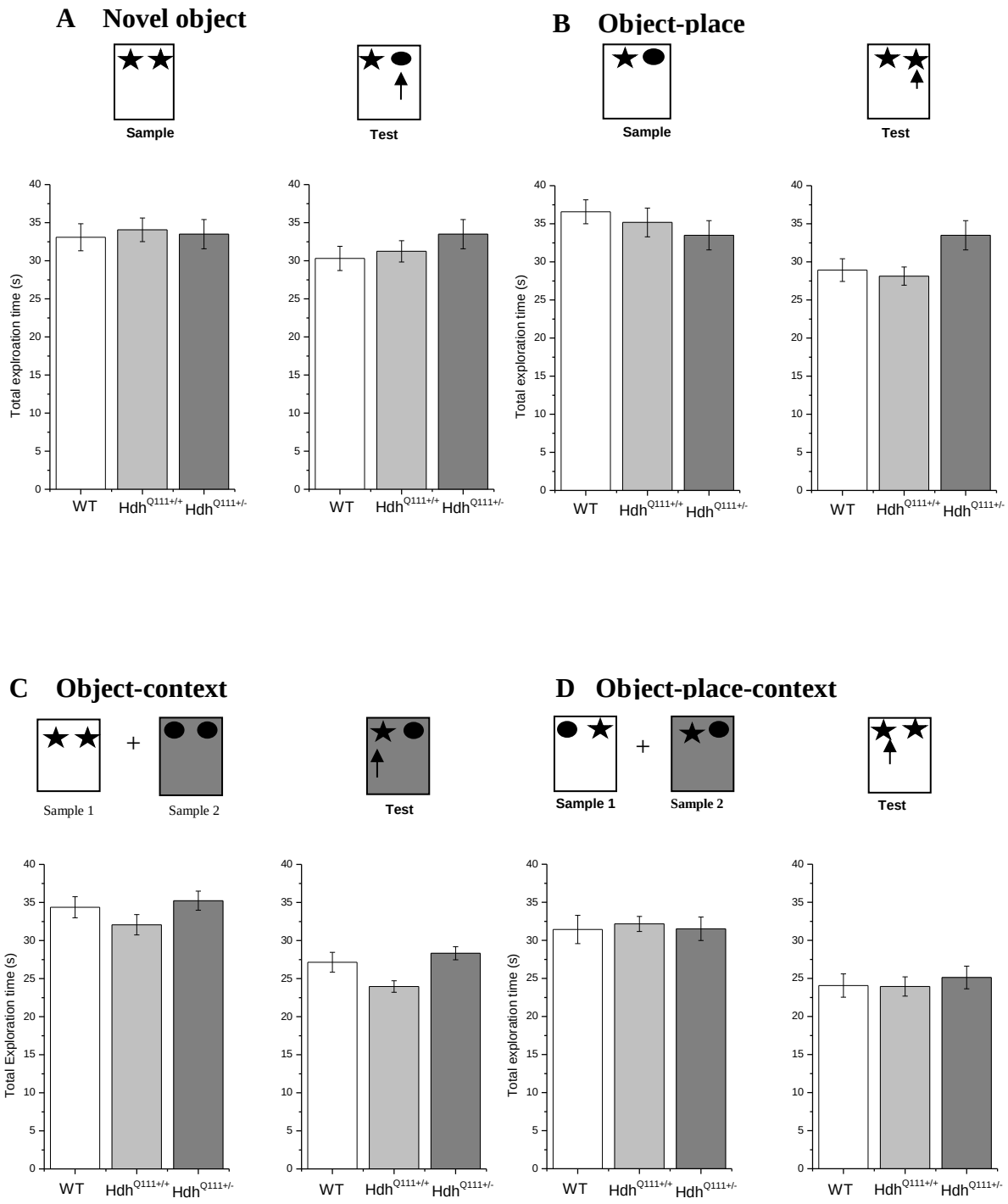



***Figure 6.4: The exploration behaviours of Hdh$^{Q111}$ mice are unaffected at 2 months.***

*For each panel: Top shows schematic representation of the task. Bottom left and right shows total exploration times of WT (n = 24), Hdh$^{Q111+/+}$ (n = 16) and Hdh$^{Q111+/-}$ (n = 24) mice (2 months) in the sample and test phases respectively. A) Novel object recognition task B) Object-place task. C) Object-context task D) Object-place-context task. All genotypes show similar exploration patterns in the sample and test phases of all four tasks (one way ANOVAs; P > 0.05).*

## 6.4: Result: Assessment of episodic memory (13 months).

In order to determine whether the cognitive deficits of $Hdh^{Q111}$ mice were progressive, the spontaneous recognition tests were also used to assess the cognition of WT ($n$ = 9), $Hdh^{Q111+/+}$ ($n$ = 10) and $Hdh^{Q111+/-}$ ($n$ = 10) mice at 13 months. The discrimination indices of the mice were evaluated. A repeated measures ANOVA was performed on discrimination scores with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs*. $Hdh^{Q111+/-}$) as the between subjects factor and task (novel-object *vs*. object-place *vs*. object-context *vs.* object-place-context) as the within subjects factor. Results indicated that there was a significant effect of genotype ($f_{(2, 24)} = 35.227$, $P < 0.05$), task ($f_{(3, 24)} = 16.686$, $P < 0.05$) and a significant task *vs.* genotype interaction ($f_{(6, 24)} = 4.544$, $P < 0.05$) (13 months). The following section will discuss the performance of $Hdh^{Q111+/+}$, $Hdh^{Q111+/-}$ and WT mice in each individual task at 13 months.

### 6.4.1: Object recognition task

WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (13 months) were initially tested in an object recognition test. Bonferroni corrected pair-wise comparisons between groups confirmed that the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice did not differ from WT mice in the object recognition task ($P > 0.05$) (Figure 6.5A). In addition, one-sample *t*-tests showed that all three genotypes explored the novel object significantly more than expected by chance. These data show that the presence of the mutant *Huntingtin* gene does not impair the ability of the mice to recognise a novel object/ remember a novel object at 13 months.

The exploration times in the object recognition task were assessed. One-way ANOVAs showed there were no significant differences in the exploration patterns of the mice in

the sample ($f_{(2, 28)} = 0.350$, $P > 0.05$) and test phase ($f_{(2, 28)} = 0.406$, $P > 0.05$) in the object recognition task (13 months) (Figure 6.6A). Although inconclusive due to enhanced performance at early ages, it was previously demonstrated that $Hdh^{Q111}$ mice displayed a mild phenotype on the rotarod from 6 months (see section 5.3.2). However, assessment of the exploration patterns in this task suggest that exploration is unaffected by the *Huntingtin* mutation at 13 months.

### 6.4.2: Object-place and object-context

The mice were then tested in the object-place and object-context tasks. $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were impaired in both the object-place and object-context task with respect to WT mice (13 months) (Figure 6.5B and 6.5C). Bonferroni corrected pair-wise comparisons between groups for each task confirmed that $Hdh^{Q111+/-}$ and $Hdh^{Q111+/-}$ mice differed from WT mice in both the object-place and object-context ($P < 0.05$) tasks (13 months). In addition, the performance of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice was similar in both these tasks ($P > 0.05$). Bonferroni corrected pair-wise comparisons indicated no significant differences in the performance of the WT mice over the object-place and object-context tasks ($P > 0.05$). In contrast, the $Hdh^{Q111+/-}$ mice performed significantly worse in the object-context ($P < 0.05$), but not the object-place task ($P > 0.05$). $Hdh^{Q111+/+}$ mice performed significantly worse in both the object-place and object-context tasks when compared to performance in the object recognition task ($P < 0.05$). Finally, one-sample *t*-tests showed that WT mice explored the novel configuration significantly more than expected by chance in both the object-place and object-context tasks ($P < 0.05$). In contrast, both the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice explored the novel configuration significantly more than expected by chance in the object-place ($P < 0.05$), but not the object-context task ($P > 0.05$). These data show that

although performance is impaired when compared to the WT, the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice are still able to identify the novel configuration in the object-place task. However, data show that the presence of the mutant *Huntingtin* gene impairs the ability of the mice to recognise a novel /remember a familiar context configuration at 13 months.

The exploration times in the associative memory tasks were assessed. One way ANOVAs showed significant differences in the exploration patterns of the three genotypes in the sample ($f_{(2, 28)} = 3.848$, $P < 0.05$) and test phase ($f_{(2, 28)} = 5.302$, $P < 0.05$) of the object-place task (13 months; Figure 6.6B). Bonferroni corrected pair-wise comparisons indicated that $Hdh^{Q111+/+}$ ($P < 0.05$), but not $Hdh^{Q111+/-}$ ($P > 0.05$) mice explored significantly less than WT mice in both the sample and test phases. The $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed similar exploration patterns throughout the object-place task ($P > 0.05$) (Figure 6.6B). However, only $Hdh^{Q111+/+}$ mice showed reduced exploration, and as $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed similar preference for the novel object-place configuration, it can be assumed that the impairment in the object-place task is not due to reduced exploration.

The exploration times in the object-context task were also assessed. One-way ANOVAs showed there were no significant difference in the exploration patterns of the mice in the sample ($f_{(2, 26)} = 2.923$, $P > 0.05$) and test phases ($f_{(2, 26)} = 1.273$, $P > 0.05$) of the object-context task (Figure 6.6C). As $Hdh^{Q111}$ mice are impaired in the object-context task in the absence of any differences in exploration, this supports the suggestion that the cognitive abnormalities in the object-place task are independent of the reduced exploratory behavior in the $Hdh^{Q111+/+}$ mice.

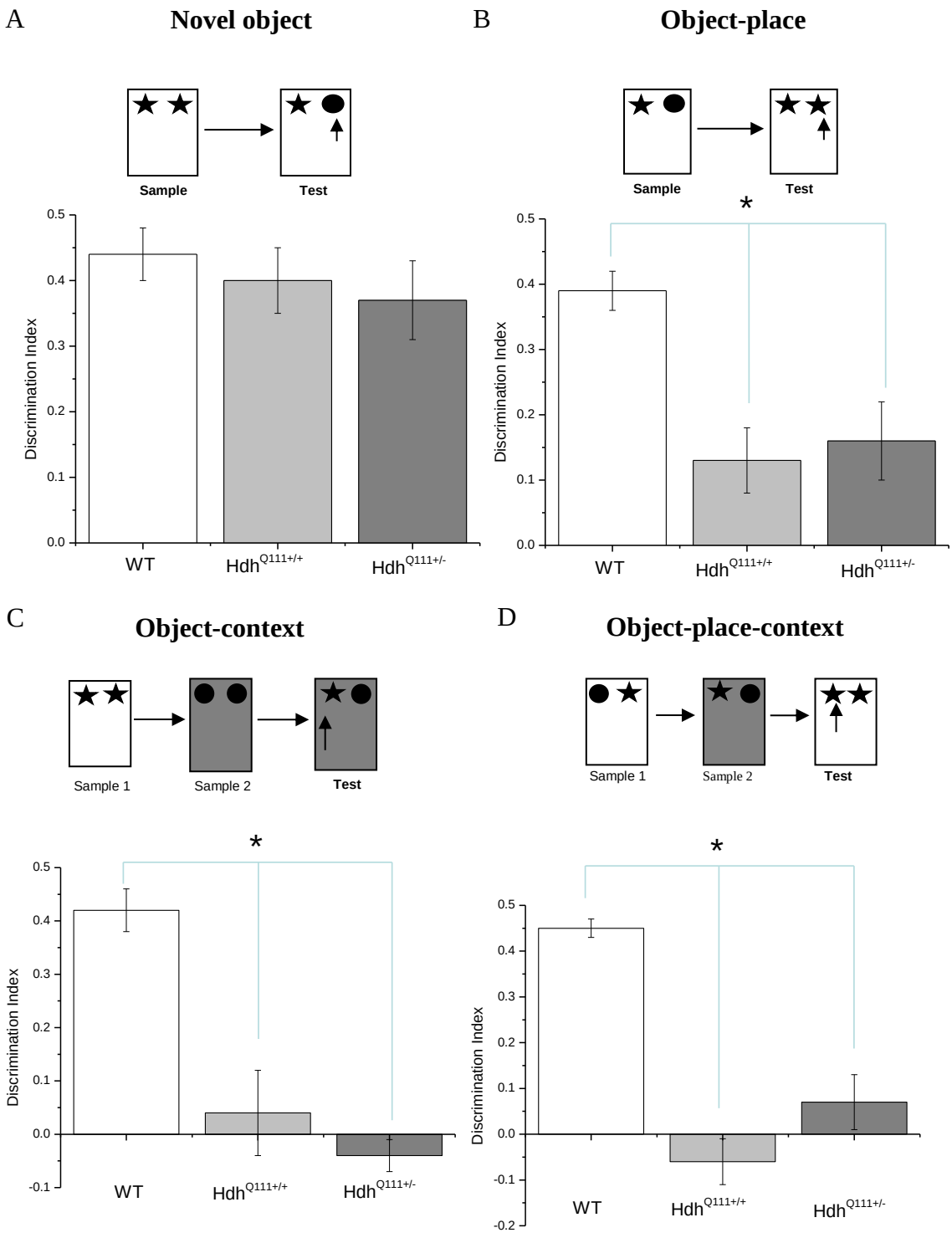



***Figure 6.5: The cognitive deficits of Hdh$^{Q111}$ mice are progressive***
*For each panel: Top shows schematic representation of the task. Bottom shows discrimination ratios. All genotypes show similar ability to identify the novel configuration in the object recognition task (P > 0.05) (A). Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice are impaired at the object-place task (B) object-context (C) and object-place-context tasks (D) when compared to WT (P < 0.05)*

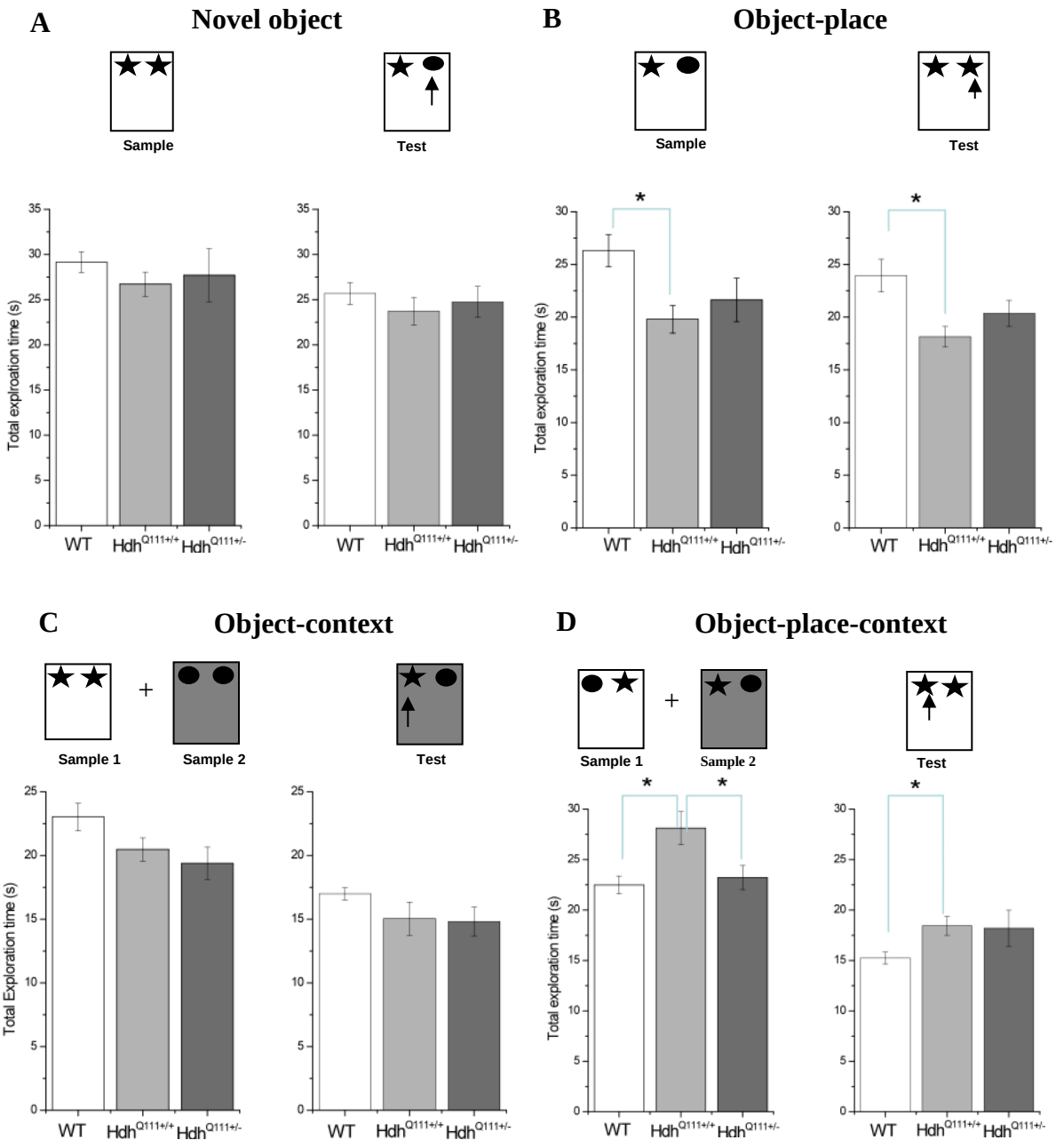



***Figure 6.6: Hdh$^{Q111}$mice do not show any major differences in exploration at 13 months***

*For each panel: Top shows schematic representation of the task. Bottom left and right shows total exploration times in the sample and test phases respectively. A) Object recognition task. All genotypes show similar exploration patterns in the sample and test phases (one way ANOVAs, P > 0.05). B) Object-place task. Hdh$^{Q111+/+}$ mice show reduced exploration in the sample and test phases (one way ANOVAs, P < 0.05) when compared to WT mice. C) Object-context. All genotypes show similar exploration in both the sample and test phases. D) Object-place-context. Hdh$^{Q111+/+}$ mice explore significantly more than WT and Hdh$^{Q111+/-}$ mice in the sample phase (one way ANOVAs, P < 0.05). Hdh$^{Q111+/+}$ mice explore significantly more than WT mice (one way ANOVAs, P < 0.05) in the test phase.*

### 6.4.3: Object-place-context task: Episodic memory

The mice were then tested for episodic memory in the object-place-context task. Bonferroni corrected pair-wise comparisons between groups for each task confirmed that the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were impaired when compared to WT mice in the object-place-context task at 13 months (both $P < 0.05$; Figure 6.5D). The $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice performed similarly in this task ($P > 0.05$). Finally, one-sample *t*-tests showed that the WT mice ($P < 0.05$), but not $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice ($P > 0.05$), explored the novel configuration significantly more than expected by chance in the object-place-context task (13 months). These results indicate that, at 13 months, the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice are impaired at the object-place-context task. This impairment is to be expected as I have shown previously that $Hdh^{Q111}$ mice are impaired at many of the component parts of episodic memory when compared to wild type at 13 months (section 6.4.2).

The exploration times in the episodic memory task were assessed. One way ANOVAs showed a significant difference in the exploration patterns of the three genotypes in the sample ($f_{(2, 28)} = 11.824$, $P < 0.05$) and test phases ($f_{(2, 28)} = 9.398$, $P < 0.05$) at 13 months (Figure 6.6D). Bonferroni pair-wise corrected comparisons showed that $Hdh^{Q111+/+}$ mice explored significantly more than WT and $Hdh^{Q111+/-}$ mice in both the sample and test phases ($P < 0.05$) of the object-place-context task. The exploration patterns of the $Hdh^{Q111+/-}$ mice did not differ from WT mice throughout the object-place-context task (both $P > 0.05$).

## 6.5 : Results : Assessment of episodic memory: Short protocol

Episodic memory was also assessed using a novel shortened protocol based on the episodic task described earlier (for more details, see section 6.2.1 and 6.2.2). This shortened protocol was used in order to determine whether similar patterns of cognitive abnormalities seen using the longer protocol could be displayed over a shorter testing period. If successful, such a behavioural test would be a valuable tool in determining when early cognitive deficits present. The discrimination indices of WT ($n$ = 14) and $Hdh^{Q111+/-}$ ($n$ = 12) mice were evaluated. A repeated measures ANOVA was performed on discrimination indices with genotype (WT *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and task (novel-object *vs.* object-place *vs.* object-context *vs*. object-place-context) as the within subjects factor. Results showed a significant effect of genotype ($f_{(2, 21)}$ = 18.667, $P < 0.05$), task ($f_{(3, 21)}$ = 9.727, $P < 0.05$) and a significant task *vs.* genotype interaction ($f_{(3, 21)}$ = 6.641, $P < 0.05$; 2 months). The next section will discuss the performance of WT and $Hdh^{Q111+/-}$ mice in each individual task, using the shortened protocol.

### 6.5.1: Object recognition task

WT and $Hdh^{Q111+/-}$ mice (2 months) were tested in an object recognition test. Bonferroni corrected pair-wise comparisons between groups confirmed that the performance of $Hdh^{Q111+/-}$ mice did not differ from WT in the object recognition task ($P > 0.05$) (Figure 6.7A). In addition, one-sample *t*-tests showed that both genotypes explored the novel object significantly more than expected by chance. These data show that the presence of the mutant *Huntingtin* gene does not impair the ability of the mice to recognise a novel object/ remember a familiar object at 2 months.

The exploration times in the non-associative memory task were assessed. One way ANOVAs showed $Hdh^{Q111+/-}$ mice explored significantly less than WT mice in both the sample ($f_{(1, 25)} = 6.087$, $P < 0.05$) and test phase ($f_{(1, 25)} = 5.163$, $P < 0.05$) (Figure 6.8A). However, as demonstrated by the discrimination indices, the reduced exploration of the $Hdh^{Q111+/-}$ mice has not affected the performance of the mice in this task.

## 6.5.2: Object-place and object-context

The mice were then tested in the object-place and object-context task. Bonferroni corrected pair-wise comparisons between groups for each task confirmed that $Hdh^{Q111+/-}$ mice did not differ from WT mice in the object-place or object-context tasks ($P > 0.05$) at 2 months (Figure 6.7B and 6.7C). The performance of $Hdh^{Q111+/-}$ mice was similar in both of these tasks ($P > 0.05$). One-sample *t*-tests showed that both genotypes explored the novel configuration significantly more than expected by chance in both the object-place and object-context tasks ($P < 0.05$). These data show that the presence of the mutant *Huntingtin* gene does not impair the ability of the mice to identify the novel/ remember the familiar novel place and context configurations at 2 months.

The exploration times were assessed. One way ANOVAs showed no significant difference in the exploration patterns in the sample ($f_{(2, 25)} = 1.166$, $P > 0.05$) and test phase ($f_{(1, 25)} = 0.018$, $P > 0.05$) of the object-place task (2 months; Figure 6.8B). In addition, one way ANOVAs demonstrated no significant differences in the sample ($f_{(1, 22)} = 0.026$, $P > 0.05$) and test phase ($f_{(1, 22)} = 0.369$, $P > 0.05$) of the object-context task (2 months) demonstrating that the huntingtin mutation does not affect exploration in either of these tasks (Figure 6.8C).

## 6.5.3: Object-place-context task: Episodic memory

The mice were then tested for episodic memory in the object-place-context task. Bonferroni corrected pair-wise comparisons between groups for each task confirmed that the performance of $Hdh^{Q111+/-}$ mice significantly differed from WT mice in the object-place-context task at 2 months ($P < 0.05$; Figure 6.7D). Bonferroni corrected pair-wise comparisons between each pair of tasks indicated that WT mice showed no significant differences between tasks (all $P > 0.05$). In contrast, the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice performed significantly worse in the object-place-context task when compared to the other tasks (both $P < 0.05$), with no difference between the object-place and object-context tasks. Finally, one-sample *t*-tests showed that WT mice ($P < 0.05$), but not $Hdh^{Q111+/-}$ mice ($P > 0.05$), explored the novel configuration significantly more than expected by chance in the object-place-context task. These data show that the presence of the mutant *Huntingtin* gene impairs the ability of the mice to identify the novel/remember the familiar object-place-configuration at 2 months. Importantly, the results of this study provide similar results to those obtained using the longer protocol (section 6.3). It is therefore possible that this novel shortened protocol could be used to assess early cognitive abnormalities when the developmental window is brief.

The exploration times in the object-place-context task were assessed. One way ANOVAs showed no significant differences in the exploration patterns in the sample ($f_{(1, 24)} = 0.015$, $P > 0.05$) and test phase ($f_{(1, 24)} = 0.262$, $P > 0.05$) of the object-place-context task (2 months; Figure 6.8D). The lack of exploration differences between genotypes further emphasises the fact that the impairments in episodic memory are not secondary to any deficits in exploration.

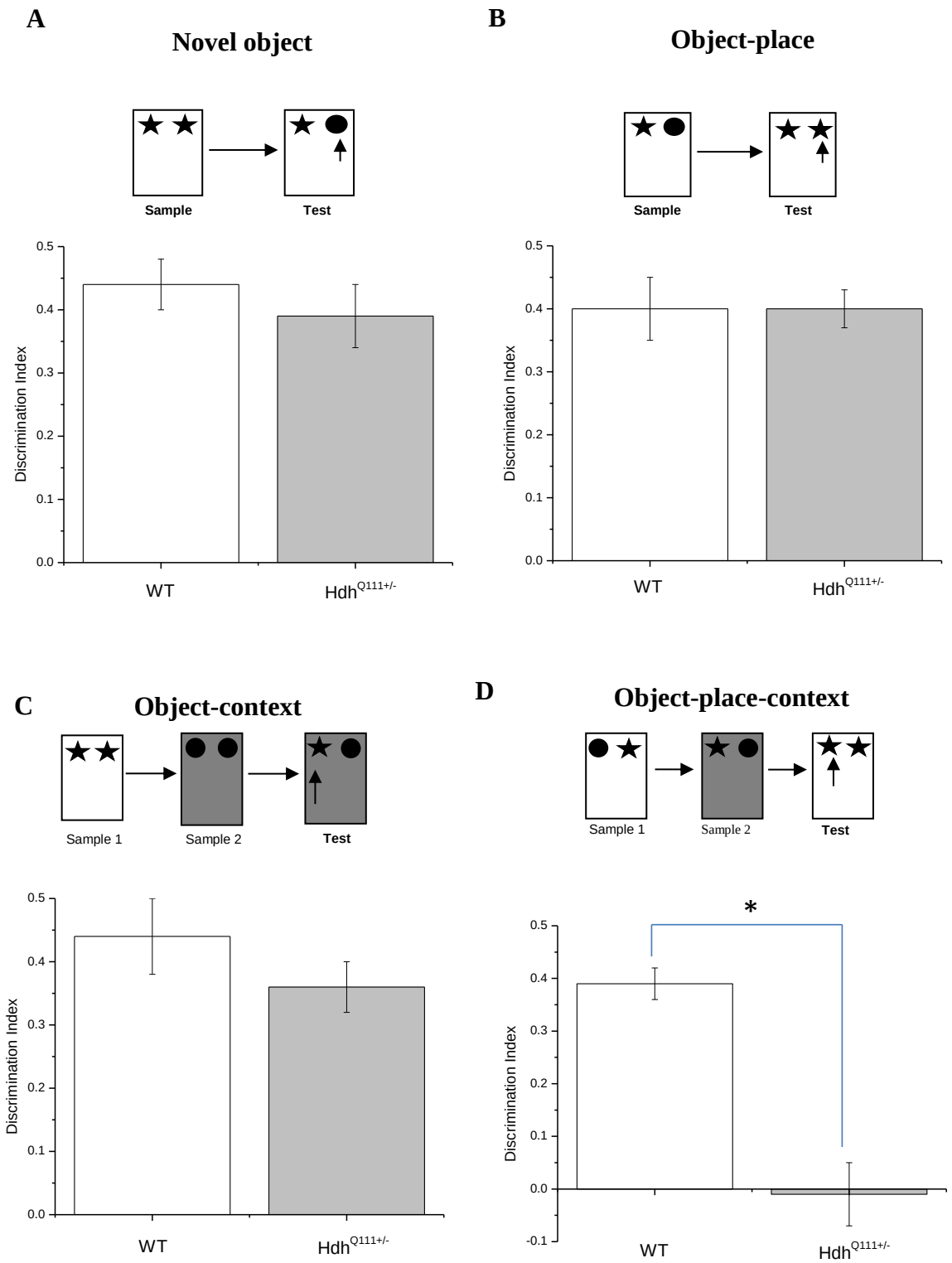



***Figure 6.7: The shortened protocol can be used to assess impairments in episodic memory in Hdh$^{Q111}$ mice at 2 months.***

*For each panel: Top shows schematic representation of the task. Bottom shows discrimination ratios of 2 month old WT (n = 14) and Hdh$^{Q111+/-}$ (n = 12) mice. Both genotypes show similar ability to identify the novel objects in the (A) novel object, (B) object-place, (C) and object-context tasks (P > 0.05). (D) Hdh$^{Q111+/-}$ mice are significantly impaired in their ability to identify the novel configuration in the object-place-context task when compared to WT mice (P < 0.05).*

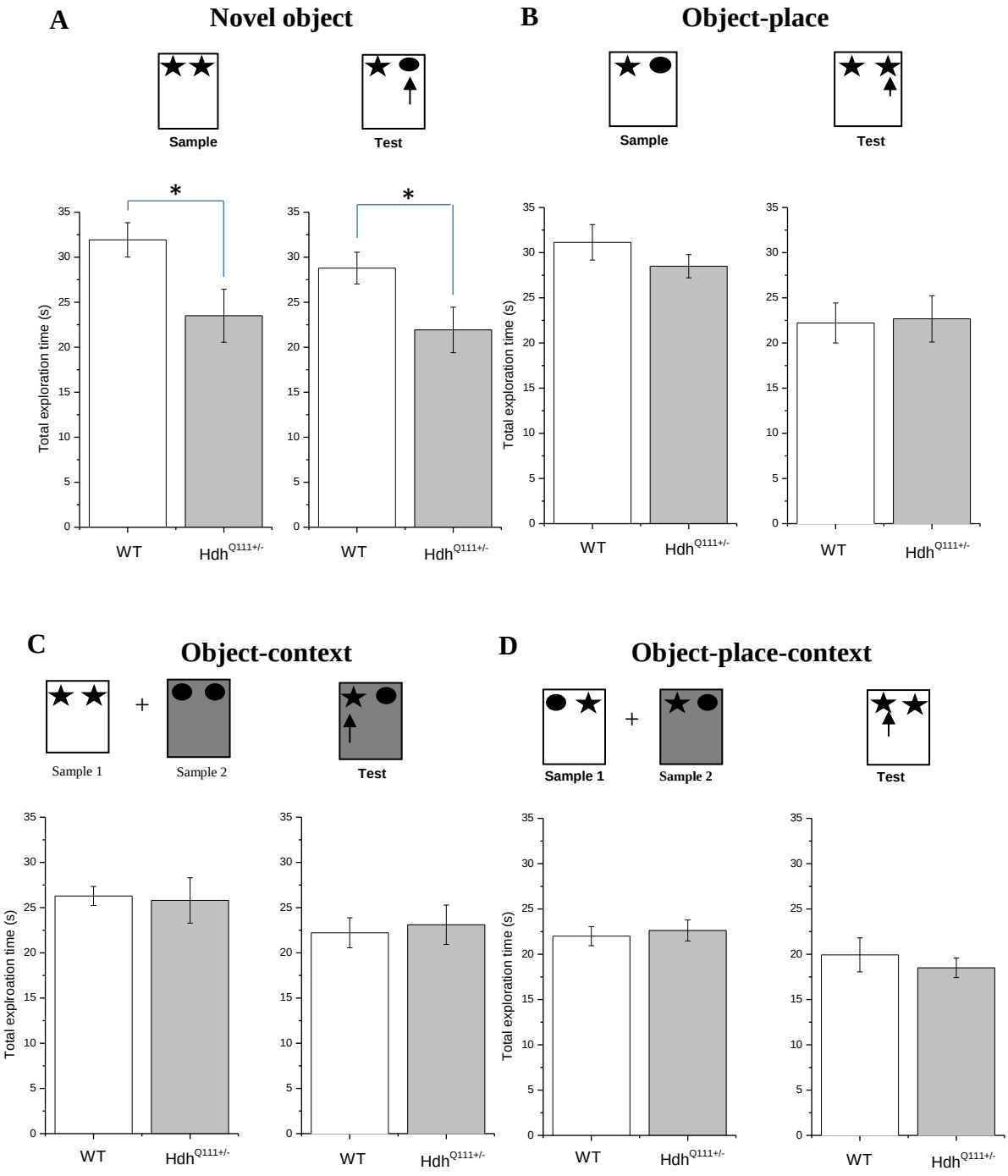



***Figure 6.8: The exploration behaviours of Hdh$^{Q111}$ mice are unaffected at 2 months***

*For each panel: Top shows schematic representation of the task. Bottom left and right shows total exploration times of the WT (n = 14) and Hdh$^{Q111+/-}$ (n = 12) mice the sample and test phases respectively. A) Object recognition task B) Object-place task. C) Object-context task D) Object-place-context task. Hdh$^{Q111+/-}$ mice show reduced exploration in the sample and test phases of the novel object task when compared to WT (one way ANOVAs, P < 0.05) Both genotypes show similar exploration patterns in the sample and test phases of the object-place, object-context and object-place-context tasks (One way ANOVAs, P > 0.05).*

## 6.6: Results: 24 hour novel object recognition task

In order to provide another cognitive test that could assess the integrity of the hippocampus in HD, the 24 hour novel object recognition test was used to assess long-term memory in $Hdh^{Q111}$ mice. Exploration times were assessed at both 5 and 10 minutes to determine whether the mice explored significantly more over 10 minutes, or whether exploration diminished after 5 minutes. One way ANOVAs demonstrated that although WT and $Hdh^{Q111}$ mice explored significantly more after 10 minutes (all $P < 0.05$), the discrimination indices calculated at 5 minutes and 10 minutes were similar (all $P > 0.05$). As a result, all the data presented in the following section represents the data obtained at the end of 10 minutes.

### 6.6.1: 24 novel object recognition task (2 months)

WT ($n = 14$), $Hdh^{Q111+/+}$ ($n = 11$) and $Hdh^{Q111+/-}$ ($n = 14$) mice (2 months) were tested in a 24 hour novel object recognition test. A one-way ANOVA was performed on discrimination scores and showed a significant effect of genotype ($f_{(2, 38)} = 79.468$, $P < 0.05$; 2 months). Bonferroni corrected pair-wise comparisons between groups confirmed that the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were impaired when compared to the WT in their ability to identify the novel object after a 24 hour delay ($P < 0.05$; Figure 6.9A). Finally, one-sample *t*-tests showed that WT mice performed significantly better than chance in the 24 hour novel object recognition task ($P < 0.05$), whereas the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice did not ($P > 0.05$).

The exploration times in the 24 novel object recognition task were assessed. One way ANOVAs showed that there was no significant differences in the exploration patterns of

the mice in either Sample 1 ($f_{(2, 38)} = 1.091$, $P > 0.05$), Sample 2 ($f_{(2, 38)} = 0.096$, $P > 0.05$) or the test phase ($f_{(2, 38)} = 0.292$, $P > 0.05$) (Figure 6.9B).

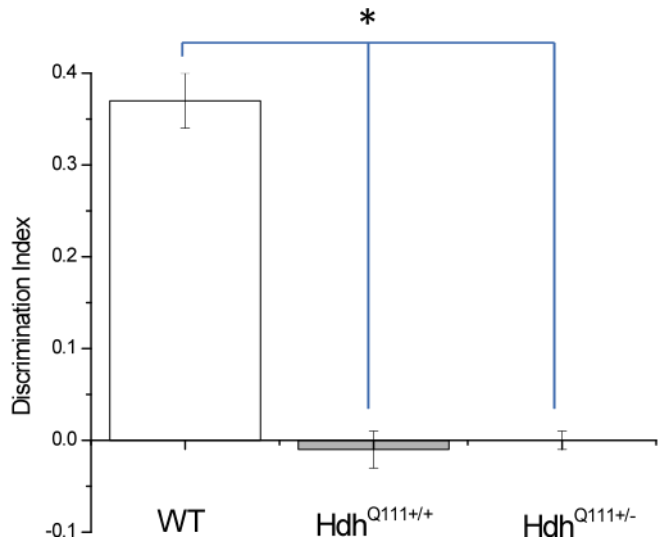
## 6.6.2: 24 hour novel object recognition task (1 month)

Following the identification of impairments in episodic memory (section 6.3) and long-term memory (section 6.5) in $Hdh^{Q111}$ mice at 2 months, it was necessary to determine the age at which the cognitive deficits appeared in the $Hdh^{Q111}$ mice. As a result, long-term memory was also assessed at the age of 1 month.

WT ($n$ = 12) and $Hdh^{Q111+/-}$ ($n$ = 12) mice (1 month) were tested in a 24 hour novel object recognition test. WT and $Hdh^{Q111+/-}$ mice showed similar performance in their ability to identify the novel object after a 24 hour delay (Figure 6.10A). A one-way ANOVA was performed on discrimination scores and showed no significant effect of genotype ($f_{(1, 23)} = 0.021$, $P > 0.05$) at 1 month. One-sample *t*-tests showed that both the WT and $Hdh^{Q111+/-}$ mice performed significantly better than expected by chance in the 24 hour novel object task ($P < 0.05$) indicating that no cognitive deficits existed in $Hdh^{Q111+/-}$ mice at 1 month.

The exploration patterns were assessed. One way ANOVAs showed that there was no significant differences in the exploration patterns of the mice in either Sample 1 ($f_{(1, 22)} = 0.062$, $P > 0.05$), Sample 2 ($f_{(1, 23)} = 1.508$, $P > 0.05$) or the Test Phase ($f_{(1, 23)} = 0.024$, $P > 0.05$) (Figure 6.10B). The lack of exploration differences between genotypes is particularly important in this experiment as this suggests that the lack of cognitive impairment in $Hdh^{Q111}$ mice is not due to altered exploratory behaviour.

A




B

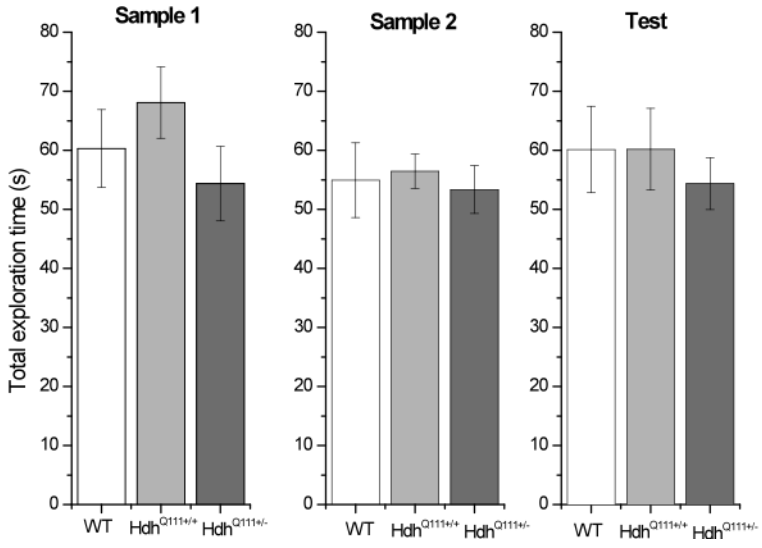
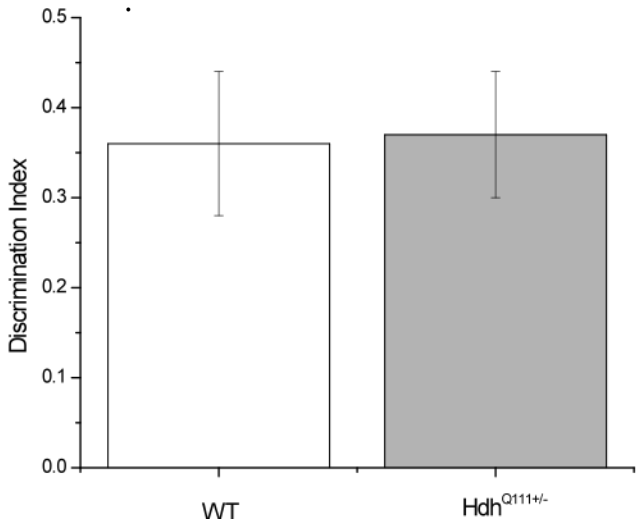



***Figure 6.9: Hdh$^{Q111}$ mice show impairments in long-term memory at 2 months***

*A) Hdh$^{Q111+/+}$ (n = 11) and Hdh$^{Q111+/-}$ (n = 14) mice are significantly impaired in their ability to identify the novel object when compared to WT (n = 14; one way ANOVA, P < 0.05) B) All genotypes show similar exploration patterns in both the sample phases and the test phase (one-way ANOVA, P > 0.05).*

A




B

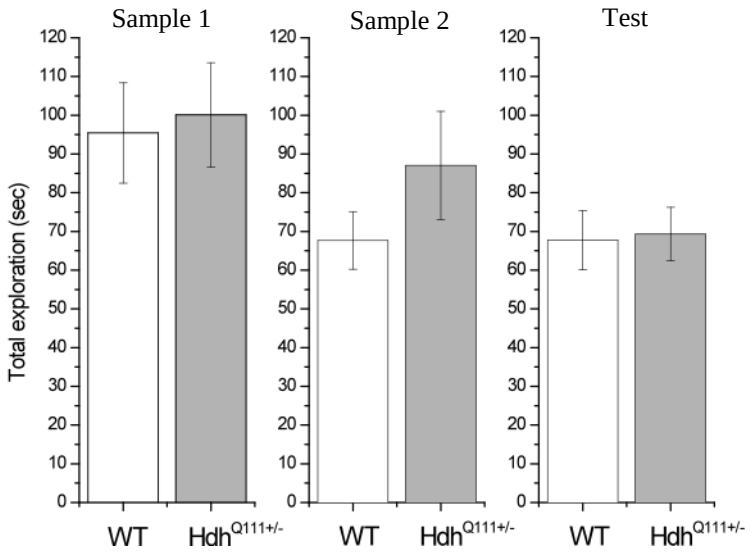



***Figure 6.10: Hdh$^{Q111+/-}$ mice show no cognitive deficits in the 24 hour object recognition task at the age of 1 month.***

*A) WT (n = 12) and Hdh$^{Q111+/-}$ (n = 12) mice have a similar performance in their ability to identify the novel object when compared to WT at 1 month (one-way ANOVA, P > 0.05) B) Both genotypes show similar exploration patterns in both the sample phases and the test phase (one-way ANOVA, P > 0.05)*

## 6.7: Discussion

The episodic memory of WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (2 months) was assessed using recognition tasks that have been previously used in rodents (Ennaceur & Delacour, 1988; Bussey *et al*., 2000; Eacott & Norman, 2004; Dere *et al*., 2005; Norman and Eacott, 2005; Langston and Wood, 2010). These tasks assess the ability of rodents to discriminate the various aspects of episodic memory, the 'what', 'where' and 'when', as defined by Tulving (1972) (see section 3.3). Studies in rodents have previously demonstrated that rats are capable of displaying episodic-like memory. 'what', 'where' and 'which' (Eacott and Norman, 2004; Langston and Wood, 2010; see section 3.4.2). However, the present study is the first time that this episodic-like memory task has been successfully utilised in mice. As many studies use mice for genetic manipulations in the development of rodent models of human diseases, this study has therefore provided an important novel tool for assessing cognitive impairments in mice. Results indicated that, at 2 months, both $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed a deficit in episodic memory as demonstrated by the impaired performance in the object-place-context task. Due to the fact that each mouse was tested in all 4 recognition tasks, I was able to confirm that the impairment in the object-place-context task in the $Hdh^{Q111}$ mice could not be attributed to secondary impairments in the recognition of object-place or object-context configurations and was not caused by a general lack of propensity to explore novelty. Therefore these data indicates that the deficit in the $Hdh^{Q111}$ mice can be specifically attributed to the object-place-context task, our model of episodic-like memory. The deficit in performance of the object-place-context task suggests that, at 2 months of age, $Hdh^{Q111}$ mice show impairments in episodic memory. Importantly, episodic memory is reported to be impaired in HD patients (Montoya *et al*., 2006; Solomon *et al*., 2007). Crucially, the cognitive

impairments in the homozygous and heterozygous $Hdh^{Q111}$ mice are similar, indicating that, reminiscent of the human disorder, only one copy of the mutant gene is necessary to produce cognitive abnormalities, further supporting the validity of the $Hdh^{Q111}$ mouse as a clinically relevant model of HD.

Due to the early cognitive impairments found in $Hdh^{Q111}$ mice at 2 months old, it was necessary to develop a novel protocol that could assess cognitive processes in a reduced time frame. To test this novel protocol, the episodic memory of $Hdh^{Q111}$ mice (2 months) was also assessed using a shortened version of the long episodic memory protocol (section 6.2.1), but instead of taking 5 weeks to complete, the mice had completed all 4 tasks in 3 days. Although this protocol has never been used in mice before, I was able to demonstrate that it could be used to produce similar results to those obtained using the longer protocol. This shortened protocol could therefore allow the cognitive abilities of mice to be examined over a shorter period of time, which is beneficial in a study where early cognitive abnormalities are being examined. In addition, a 24 hour novel object recognition task was used to demonstrate that the $Hdh^{Q111}$ show no cognitive impairments at the age of 1 month. With such a narrow developmental window in which the cognitive deficits become apparent, both the shortened version of the episodic memory task and 24 hour novel object recognition task would therefore be useful in order to determine the specific age at which the cognitive deficits occur in the $Hdh^{Q111}$ mouse model of HD, and also to assess the effectiveness of potential cognitive therapies. Although the 24 hour novel object recognition task is not a model of episodic memory, previous studies have indicated a role of the hippocampus in long-term novel object recognition (Clark *et al*, 2000; Broadbent *et al*, 2004; Ainge *et al*., 2006 – see section 3.4.1). As a result, the 24 hour

novel object task was used in order to provide a second, efficient cognition test that could assess the integrity of the hippocampus in the early stages of HD.

The lack of a short-term memory impairment in the performance of the $Hdh^{Q111}$ mouse (2 months) in the object recognition task agrees with a previous study which indicated that, at 12 and 14 weeks, the R6/1 transgenic model of HD shows similar preference for the novel object when compared to control animals (Nithianantharajah *et al*., 2008). However, in contrast to the lack of impairment of the $Hdh^{Q111}$ mice in the object-place task (2 months), R6/1 mice show impairments in a test used to assess location memory (12 – 14 weeks; Nithianantharajah *et al*., 2008). These contrasting results could perhaps be explained by protocol differences (see section 3.4.3). However, the contrasting results could also be explained by the fact that the R6/1 displays a more severe phenotype than the $Hdh^{Q111}$ mouse (see section 1.2.1.2). This suggestion is supported by the observation that the $Hdh^{Q111}$ shows deficits in the object-place task by the age of 13 months. Neuronal cell loss is evident in the hippocampus and it is thought that it is the changes within the hippocampus that underlie the cognitive abnormalities that characterise the early stages of HD (Rosas *et al.,* 2003). It is perhaps the case that, in the early stages of the disorder, deficits only result in tasks that recruit the hippocampus. Rat lesions studies assessing the differential role of the hippocampus in memory could perhaps provide an insight behind the cognitive deficits in $Hdh^{Q111}$ mice at 2 months (Ennaceur *et al*., 1997; Clark *et al*., 2000; Mumby *et al*., 2002; Broadbent *et al*., 2004; Eacott and Norman, 2004; Ainge *et al.*, 2006; see section 3.4). Lesion studies have shown that the hippocampus is only required for the integration of object, place and context configurations, as memory for the individual components of episodic memory remain intact following lesions of the fornix/ hippocampus (Eacott and Norman, 2004;

Langston and Wood, 2010; section 3.4.2 and 3.4.3). In addition, lesion studies have indicated that the hippocampus could be important in novel object recognition at long delays (Clark *et al*, 2000; Broadbent *et al*, 2004; Ainge *et al*., 2006 – see section 3.4.1). The lack of impairment in the 24 hour novel object recognition task at 1 month therefore suggests that the hippocampus is functioning normally in the $Hdh^{Q111}$ mouse early in development.

The long-term memory of $Hdh^{Q111}$ mice was assessed at 2 months. In contrast to the results of the short-term novel object recognition task, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were unable to identify the novel object following a 24 hour retention time, whereas WT animals had no problem successfully completing this task. Therefore, in order to gain a better understanding of the contrasting results in the short- and long-term recognition tasks, a task could be designed in which short-term recognition memory (2 minute retention) and long-term recognition memory (24 hour retention) protocols could be more accurately compared *i.e.* with identical sample and test times. In addition, the retention times could also be varied in attempt to determine at which point at which recognition memory becomes impaired. If it is indeed the case that abnormalities within the hippocampus contribute to the early cognitive deficits in HD, it would be beneficial to assess the point at which recognition memory recruits the hippocampus, thus resulting in the development of cognitive impairments in HD.

An alternative explanation could also perhaps explain the contrasting results. Previous studies have demonstrated that patients of HD are unable to recall information (Butters and Grady, 1977; Butters *et al*., 1978; Caine *et al*., 1978), and several studies have shown that although human patients are able to store and retain new information, they

are unable to retrieve the information at a later date (Butters *et al*., 1983; Butters *et al*., 1985; Moss *et al*., 1986; Delis *et al*., 1991; Lundervold *et al*., 1994). If this is indeed the case, it is possible that in the test of short-term recognition memory, the $Hdh^{Q111}$ mice are using working memory to identify the novel object. However, when longer retention intervals are used, *i.e.* 24 hours, there is requirement for the storage of the encoded information, which the $Hdh^{Q111}$ mice are subsequently unable to retrieve, thereby displaying a cognitive deficit at longer retention intervals. In the case of the short-term cognitive impairment in object-place-task, as a model of episodic memory this task should recruit the hippocampus. Importantly, rat lesion studies have demonstrated that this is indeed the case (Eacott and Norman, 2004; Langston and Wood, 2010 – see section 3.4.2). It is possible that the deficits in episodic memory occur as a result of damage to the hippocampus in the early stages of HD, thus explaining why the short-term recognition memory of object-place and object-context is intact.

The results from the assessment of cognitive processes in the older animals suggest that the early cognitive deficits present in the $Hdh^{Q111}$ mice are progressive, perhaps spreading to different regions of the brain by the age of 13 months. It would therefore be beneficial to know which brain regions are involved in each of the tests used to assess short-term recognition memory. Lesion studies have indicated a role of the perirhinal cortex in the object recognition task (Ennaceur *et al.*, 1996., Bussey *et al.*, 2000), but not the object-place memory (Ennaceur *et al*., 1997; Eacott and Norman, 2004). In addition, lesions of the perirhinal cortex only cause relatively mild, delay-dependent impairments of object–context memory (Norman and Eacott, 2005). However, lesions of the postrhinal cortex impair memory for object–context associations (Norman and Eacott, 2005). In addition, unpublished data has

demonstrated that the entorhinal cortex is involved in the processing of object-context configurations (Ainge and Langston, 2010). In agreement, the entorhinal cortex is one of the earliest brain regions to be affected in Alzheimer's disease (Van Hoesen *et al*., 1991; Gomez-Isla *et al*., 1996; Du *et al*., 2001; Mega, 2001) so it is possible that the cognitive deficits in the $Hdh^{Q111}$ mouse could also link to the abnormalities within the entorhinal cortex.

In order to determine which brain regions are specifically activated during each test it is possible to examine Fos protein expression within specific brain regions. The expression of *c-fos*, an early gene-product marker of Fos, can be used as an indirect marker of neuronal activity as expression reflects an intracellular state of cells that varies primarily as a result of recent activation by intercellular signals. If, for example, the perirhinal/ postrhinal or entorhinal cortices are involved in any of the short-term spontaneous recognition memory tasks in mice, that particular brain region would show an upregulation of *c-fos* mRNA expression (Dragunow and Faull, 1989; Day *et al*., 2008; Van Elzakker *et al*., 2008). Following these studies, if any brain regions show Fos expression, lesion studies specifically targeting these regions could be performed in control animals in order to assess which brain regions are critical for cognitive processing of the object-place and object-context tasks.

# 7: Experiment 3: Characterisation of the electrophysiological phenotype of the $Hdh^{Q111}$ mouse

Hippocampal synaptic plasticity has been proposed as a correlate of learning and memory (see section 2.3). Impairments in synaptic plasticity have been demonstrated in mouse models of HD prior to the appearance of an overt motor phenotype (Usdin *et al.*, 1999; Murphy *et al.*, 2000; Lynch *et al.*, 2007; Simmons *et al*., 2009). Subsequently, it is possible that changes in synaptic plasticity in the $Hdh^{Q111}$ mouse could underlie cognitive deficits seen in HD (Lione *et al.*, 1999; Mazarakis *et al.*, 2005; Van Raamsdonk *et al.*, 2005; see section 6.1). The following section will therefore discuss the hippocampal synaptic plasticity deficits in the $Hdh^{Q111}$ mouse model of HD.

## 7.1: HD and deficits in LTP

The following section will introduce synaptic plasticity deficits that have been demonstrated in mouse models of HD (Usdin *et al.*, 1999; Murphy *et al.*, 2000; Lynch *et al.*, 2007; Simmons *et al*., 2009). The synaptic physiology of hippocampal slices derived from wild type and heterozygous mutant 80 CAG mice was assessed (8 – 14 months; for more information on the 80 CAG mouse model, see section 1.2.2.1) (Usdin *et al*., 1999). Although baseline synaptic function (*i.e.* control fEPSPs) was similar to that of control mice, LTP was significantly reduced for the mutant animals when compared to control (Usdin *et al*., 1999). However, the LTP deficit in this model of HD could be rescued by applying a stronger LTP induction protocol. Therefore, Usdin *et al.* (1999) speculated that instead of disabling the LTP mechanism, the huntingtin mutation in the 80 CAG mouse increased the threshold for LTP induction.

In addition, a study by Lynch *et al.* (2007) investigated synaptic plasticity in pre-symptomatic homozygous $Hdh^{Q92}$ and $Hdh^{Q111}$ knock-in mice (2 months; for more information on the $Hdh^{Q92}$ and $Hdh^{Q111}$ mice, see Section 1.2.2.2). Basal synaptic properties were comparable for WT, $Hdh^{Q92}$ and $Hdh^{Q111}$ mice. Furthermore, paired-pulse facilitation did not differ between the genotypes, suggesting that there are no presynaptic abnormalities in this mouse model. However LTP, assessed 60 minutes post-theta burst stimulation (TBS), was significantly reduced in the slices from the knock-in mice (Lynch *et al.*, 2007).

A study by Murphy *et al.* (2000) demonstrated reduced synaptic plasticity in the R6/2 mouse model of HD (for more information on the R6/2 mouse, see section 1.2.1.1). Although basal synaptic transmission was normal, LTP following a high-frequency conditioning stimulus was significantly reduced at the CA1 synapses of hemizygous R6/2 mice at all ages from 5 – 18 weeks. The deficits in synaptic plasticity in the R6/2 mouse are also associated with cognitive deficits as demonstrated by impaired spatial memory at 7 weeks. Similarly, LTP deficits and impairments in long-term memory have been demonstrated in homozygous $Hdh^{Q140}$ mice (8 weeks) (Simmons *et al.*, 2009; for information on the $Hdh^{Q140}$ mouse, see section 1.2.2.3). The results of these studies therefore indicate that dysfunctional synaptic transmission may underlie the cognitive deficits seen pre-symptomatically in HD (Usdin *et al.*, 1999; Simmons *et al.*, 2009).

Collectively, these studies have shown that mutant huntingtin severely impairs the stabilisation of LTP (Usdin *et al.*, 1999; Murphy *et al.*, 2000; Lynch *et al.*, 2007; Simmons *et al.*, 2009). This section will assess basal synaptic transmission and LTP in the $Hdh^{Q111}$ mouse model of HD in order to detect early hippocampal abnormalities,

thereby allowing a further understanding of the relationship between cognitive deficits and synaptic plasticity in HD.

## 7.2: Methods

### 7.2.1: Hippocampal brain slice preparation

Adult male WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (2 months) were sacrificed by cervical dislocation in accordance with Schedule 1 of the UK Animals (Scientific Procedures) Act 1986. The brain was dissected was immersed in oxygenated (95% $O_2$, 5% $CO_2$) artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 3 KCl, 1 $MgSO_4.7H_2O$, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 1 $CaCl_2$ and 10 D-glucose. The cerebellum and lateral portion of the temporal lobes were removed, and the brain was halved down the midline and glued to a metal plate. Sagittal slices (400 µm) were cut using feather blades (Agar Scientific, Essex, UK) while mounted on a Vibratome (Intracel, Series 1000, Royston, Hert, UK), and continuously submerged in aCSF. Sagittal hippocampal slices were cut from each of the bisected hemispheres. Such slices were then placed on a submerged nylon mesh in an incubation chamber, filled with oxygenated aCSF at room temperature for at least an hour before use in electrophysiological recording.

### 7.2.2: Solution application

A single slice was transferred to a submerged recording chamber (Scientific Systems Design, Mississauga, Ontario, Canada) to obtain electrophysiological recordings. Oxygenated aCSF solution, was held at a bath temperature of 31 - 32ºC by a temperature controller (Digitimer, Research Instruments, Hertfordshire, UK), using an oxygenated water chamber to circulate warm water beneath the slice. The slice was

oxygenated in the perfusion system with aCSF (flow rate ~2 ml/min). Gravity feed was manipulated to maintain a constant flow rate and a Gilson pump (AD Instruments, model Minipuls evolution, Calgrove, Oxfordshire, UK) was used for suction.

### 7.2.3: Generation of field excitatory postsynaptic potentials (fEPSPs).

To monitor basal synaptic transmission a bipolar stimulating electrode, either hand-made from twisted Teflon-coated tungsten wire (Advent research materials, Ltd, Eynsham, Oxfordshire, UK) or a commercial electrode (World Precision Instruments, Florida, USA) was used to stimulate the Schaffer collateral-commissural pathway from area CA3 to the CA1 region of the hippocampus. Stimulation of these presynaptic fibres results in the release of glutamate at the synapse between the axons of the CA3 region and dendrites of the CA1 neurons, causing a synchronous postsynaptic depolarization, resulting from the activation of ionotropic receptors (primarily AMPA receptors) and influx of positive ions into the postsynaptic terminal (Amaral and Lavenex, 2007). The stimulus was delivered to the slice every 30 seconds to record dynamic changes in fEPSPs and this was controlled by a constant current isolated electronic stimulator (Digitimer Ltd, model DS2, Hertfordshire, UK) which was electrically isolated to prevent interference from the mains electrical noise. The stimulatory current was adjusted to produce a response with a slope measurement that was 40% of the maximum population spike-free response. fEPSPs were recorded using an aCSF-filled glass borosilicate microelectrode, (Kind precision glass, Inc, Claremont, USA), which had been pulled to the desired tip resistance of less than 5 MΩ (inner diameter of 0.69 mm) by a vertical electronic puller (Narishige, Japan, model PP-830) and placed in the apical dendrite layer of CA1 pyramidal cells. Within the recording electrode was a silver chloride wire (Advent research materials, Ltd, Eynsham, Oxfordshire, UK) and

this, together with a ground electrode of silver chloride wire, was attached to an isolated differential amplifier where the signal was amplified and filtered (3 kHz – 10 kHz) (Warner Instrument Corporation, Connecticut, USA). The output from the amplifier was fed through an oscilloscope (Tektronix, Oregon, USA) and digitised through an acquisition board (BNC-2090, National Instruments, Berkshire, UK). Both stimulating and recording electrodes were positioned using manipulators under visual guidance through a microscope (Olympus, SZ30, Essex, UK).

### 7.2.4: fEPSP analysis

Analysis of fEPSPs was performed using WinLTP software (Anderson, www.winltp.com). The WinLTP software provides a visual representation of the evoked fEPSP and can also be used to alter the stimulation protocol *(e.g.* the number, or the duration of pulses) or the parameters of the fEPSP recording (*e.g.* the length of recording, or amplitude and slope detection values). The fEPSP has a number of characteristic features (Figure 7.1) and measurement of amplitude and slope of the fEPSP can be taken to give an indication of synaptic activity within the neuronal population. Analysis of the slope of the fEPSPs was used as a measurement of the strength of synaptic transmission and was measured from 30% - 70% of the peak amplitude. The percentage increase in fEPSP slope when compared to baseline was calculated offline using Origin 7 software (OriginLab Corporation, Northhampton, MA, USA) and this was used to quantify the magnitude of LTP.

### 7.2.5: Input-output function

Basal synaptic transmission was studied with an input-output curve. This is a measure of synaptic recruitment, which increases with the input stimulus due to the activation of

a greater population of neurons. Input-output curves were generated using increasing stimulus intensities and the slope of each of the evoked fEPSPs was measured. The stimulus was initially set a point at which no fEPSP current was evoked and was then increased at 10 μA increments. For each stimulation intensity three stable slope recordings were measured at 30 second intervals and the responses were subsequently averaged. The slope of the fEPSP was then plotted as a function of stimulus strength. The curve was completed when a plateau phase was reached and the slope of the fEPSP remained similar at several increasing stimulus intensities.

### 7.2.6: Paired-pulse facilitation

Paired-pulse facilitation (PPF) was recorded at inter-stimulus intervals of 20, 50, 100, 200, 300, 400 and 500 ms. PPF was plotted as a paired pulse ratio, calculated by dividing the amplitude of the second fEPSP by the amplitude of the first fEPSP *versus* inter-stimulus interval. Values of greater than 1 indicate facilitation. Three steady fEPSP slope responses were recorded at 30 second intervals for each inter-stimulus interval and the measurements were subsequently averaged.

### 7.2.7: LTP induction

There are a number of stimulation protocols which have been used to induce LTP in the hippocampal slice preparation. The theta burst protocol is characterised by an inter-burst interval of 200 ms and is considered to be physiologically relevant as similar firing patterns exist in the hippocampus during learning (Otto *et al*., 1991). A 10 minute stable baseline recording of control fEPSPs was obtained after which LTP was induced, by the same stimulating electrode by applying a 4 pulse TBS (4-TBS) protocol in which 10 bursts of stimulation pulses were delivered at a frequency of 5 Hz; each burst

containing 4 pulses at 100 Hz. The fEPSP measurements were obtained for an additional 60 minutes. The magnitude of LTP was assessed between 40 – 50 minutes post-TBS (for more details on analysis and statistics, see section 7.2.8 and 7.2.9).

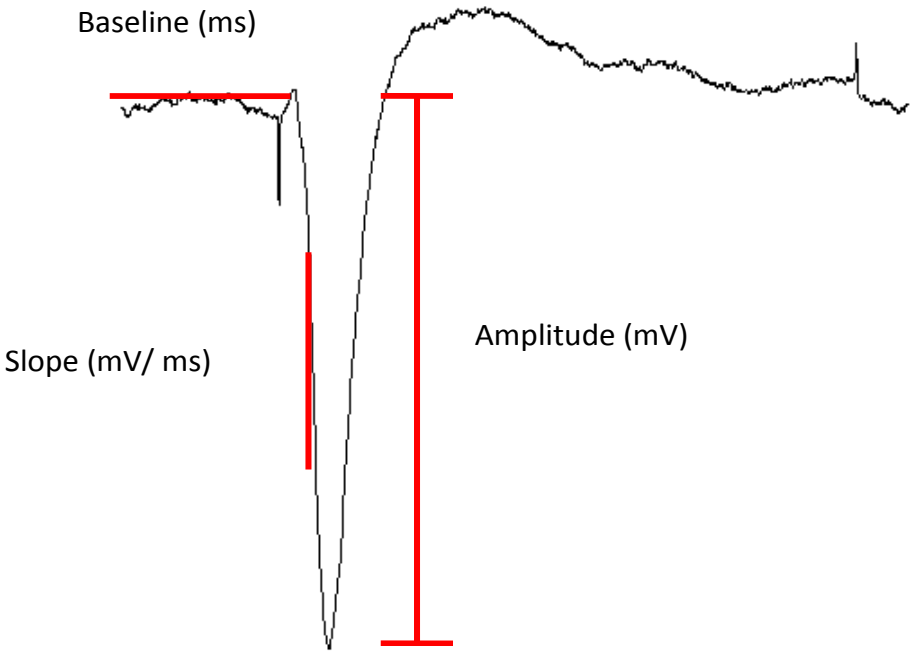



***Figure 7.1: A field extracellular postsynaptic potential (fEPSP).*** *The amplitude is calculated as the measurement from the baseline to the peak of the fEPSP, while the slope is calculated between 30-70% of the amplitude. The stimulus artifact appears as a brief spike immediately before the fEPSP. The fEPSP represents the simultaneous depolarisation of a population of CA1 pyramidal neurons, which is recorded as a negative voltage deflection due to the flow of positive ions away from the recording electrode into the postsynaptic terminals.*

## 7.2.8: Measurement of LTP

Following a TBS there is an immediate increase in both the slope and amplitude of the fEPSP. This enhanced response decreases rapidly in the first 5 minutes after LTP induction, causing a steep decrease in the slope and the amplitude of the fEPSP. A plateau phase is reached where the slope and amplitude of the fEPSP both reach a steady maintained state, which is enhanced in comparison to the original control fEPSPs. Experiments that did not follow this pattern, *i.e*. decayed below baseline, were discarded. LTP was measured using the average potentiation of the plateau phase, taken at 50 minutes following the 4-TBS. The mean value of each individual slice was calculated and then for the group as a whole. The average potentiation determined from all fEPSPs between 40 – 50 minutes post TBS was used for statistical comparison between the groups. In order to allow experiments to be accurately pooled together, LTP was plotted as a normalised measurement of control fEPSP slope values.

## 7.2.9: Statistics and analysis

All graphs of electrophysiological experiments were created using Origin 7 software (OriginLab Corporation, Northhampton, MA, USA). Input-output curves were compared using a one-way ANOVA. PPF and LTP measurements were analysed by a repeated measures ANOVA. The analysis used is indicated in the text where appropriate. Significance was noted at the levels of $P < 0.05$. Data is presented as mean ± SEM and $n$ = number of slices.

# 7.3: Results

## 7.3.1: Input/ output function

Normalised input-output curves were generated and compared for WT mice, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (2 months; age range 59 – 74 days – hereafter referred to as 2 months old). A normalised input-output curve, where the maximum fEPSP slope was set to 100%, was generated. Results showed that there were no significant differences in the normalised input-output curve in 2 month old WT ($n$ = 8), $Hdh^{Q111+/+}$ ($n$ = 9) or $Hdh^{Q111+/-}$ ($n$ = 8) mice (One-way ANOVA: $f_{(2, 51)} = 0.213$, $P > 0.05$; Figure 7.2A).

## 7.3.2: Paired-pulse facilitation

Paired-pulse facilitation was compared between 2 month old WT mice, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice. In all experiments, the maximum facilitation was observed at an inter-stimulus interval of 50 ms. In WT mice ($n$ = 8), the paired-pulse ratio at 50 ms was 1.78 ± 0.06, while in $Hdh^{Q111+/+}$ ($n$ = 9) and $Hdh^{Q111+/-}$ ($n$ =6) was 1.8 ± 0.06 and 1.73 ± 0.04 respectively. Subsequent statistical analysis revealed that the paired-pulse ratio is similar in all 3 genotypes (Repeated measures ANOVA: $f_{(2, 23)} = 0.04$, $P > 0.05$; Figure 7.2B).

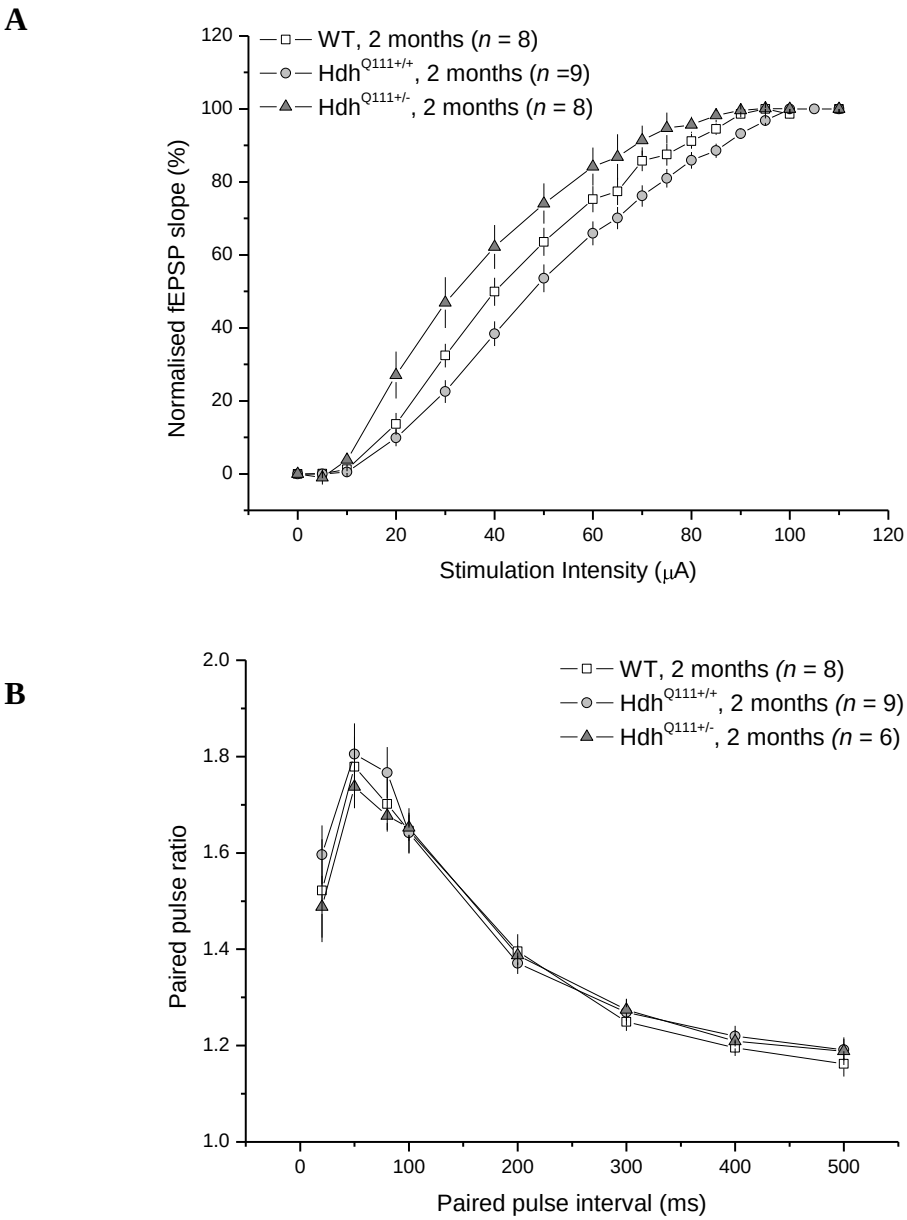



***Figure 7.2: Input/output function and PPF is normal in the Hdh$^{Q111}$ mice (2 months)***

*A) Input/output function. The fEPSP slope was measured at a range of stimulus intensities and then normalised for WT (n = 8), Hdh$^{Q111+/+}$ (n = 9) and Hdh$^{Q111+/-}$ (n = 6) mice. The input/output function was similar in all 3 genotypes (One-way ANOVA; P > 0. 05). B) Paired-pulse facilitation. The paired-pulse facilitation was calculated at a range of inter-stimulus intervals (20, 50, 80, 100, 200, 300, 400 and 500 ms) in WT (n = 8), Hdh$^{Q111+/+}$ (n = 9) and Hdh$^{Q111+/-}$ (n = 8) mice. Paired-pulse facilitation was similar in all 3 genotypes (Repeated measures ANOVA; P > 0.05).*

## 7.3.3: LTP is impaired in the $Hdh^{Q111}$ mouse model of HD (2 months)

For hippocampal slices derived from WT mice (*n* = 14), a 4-TBS induced a robust magnitude of LTP, (93 ± 16 % increase *cf.* control at 50 minutes post-TBS). Similarly, for brain slices derived from either $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice a 4-TBS induced LTP, but for both genotypes the magnitude was considerably reduced *c.f.* WT ($Hdh^{Q111+/+}$ = 50 ± 8 % increase, *n* = 9; $Hdh^{Q111+/-}$ = 28 ± 6 % increase, *n* = 15; Figure 7.3).

These data illustrates that, at 2 months, LTP is significantly impaired in both $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ brain slices, when compared to WT (repeated measures ANOVA, effect of genotype; $f_{(2, 33)} = 8.558$, $P < 0.05$). Bonferroni corrected pair-wise comparisons between groups confirmed that potentiation of the fEPSP slope 50 minutes post-TBS was significantly reduced in both $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ brain slices when compared to WT ($P < 0.05$), but that there was no significant difference ($P > 0.05$) between heterozygous and homozygous HD mice in this respect (Figure 7.3).

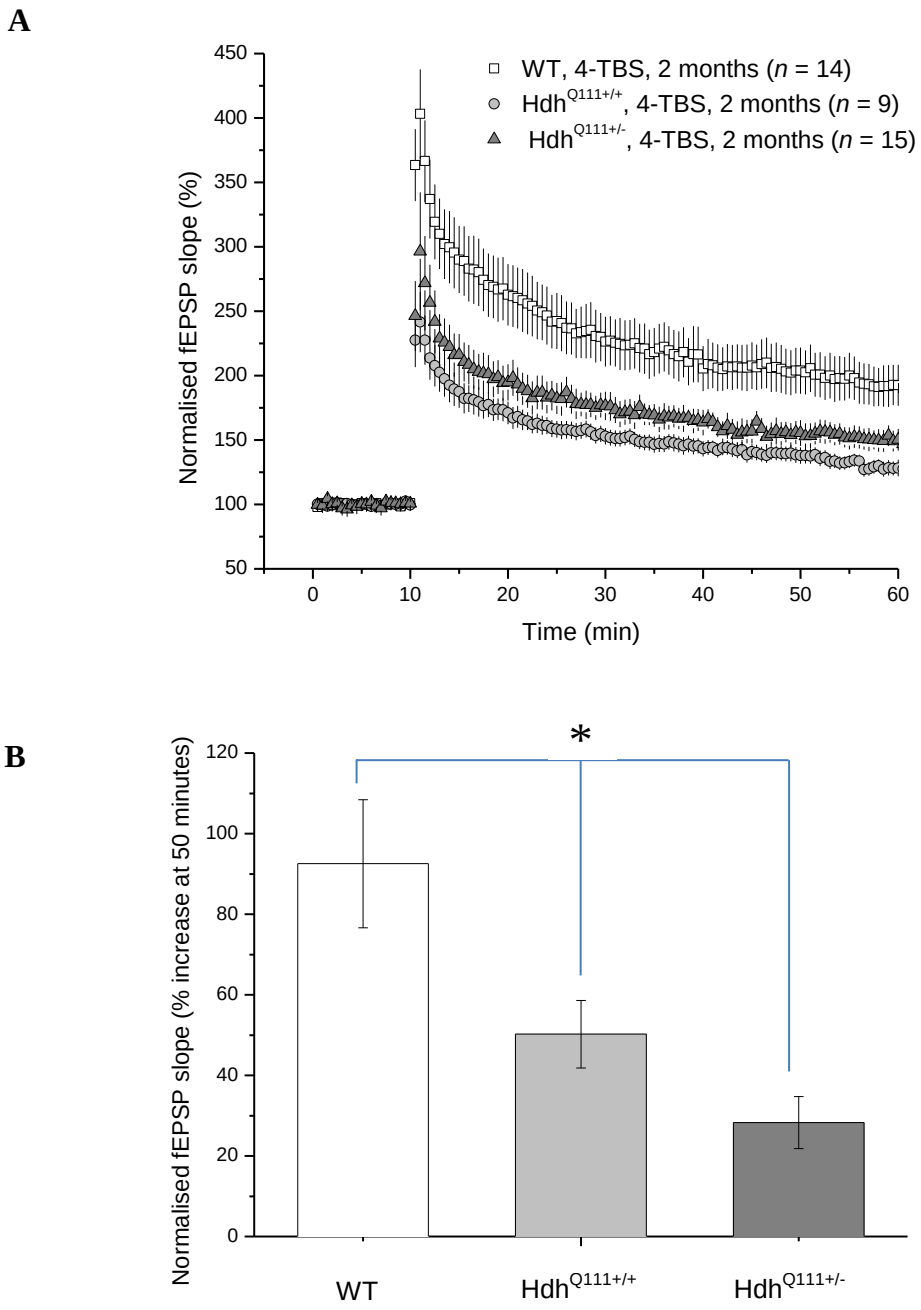



***Figure 7.3: LTP is impaired in both homozygous and heterozygous 2 month old Hdh$^{Q111}$ mice***

*A) The time course of the changes in the slope of the fEPSP after the induction of LTP by a 4-TBS, applied after the establishment of a 10 minute control baseline. The fEPSP slope is plotted as the percentage change against time and expressed as a pooled mean (± SEM). B) A 4-TBS induced robust LTP in WT slices (93 ± 16% increase; n = 14), but a significantly reduced magnitude of LTP in Hdh$^{Q111+/+}$ (50 ± 8 % increase) and Hdh$^{Q111+/-}$ (28 ± 6 % increase) slices (P < 0.05). LTP deficits are similar in Hdh$^{Q111+/+}$ and Hdh$^{Q111+/-}$ mice (P > 0.05). Error bars indicate SEM. n values indicate number of slices.*

## 7.4: Discussion

These results indicate that the properties of fEPSPs are similar in WT, WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ hippocampal CA1 neurons. The similarity in the input/output function suggests that $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ slices have approximately the same density of synapses as control and these synapses also respond similarly to a single stimulus. In addition, the paired-pulse ratios did not differ between the genotypes, suggesting no impairment of presynaptic function. The lack of any impairments in the fEPSP properties of $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ CA1 neurons agrees with previous studies which have demonstrated that PPF in hippocampal slices derived from $Hdh^{Q92}$ and $Hdh^{Q111}$ mice (Lynch *et al*., 2007) and R6/2 mice (Murphy *et al*., 2000) does not differ from those of WT (section 7.1).

Although basal excitatory synaptic transmission was normal in $Hdh^{Q111}$ mice, due to the cognitive deficits that are associated with HD, the effect of the huntingtin mutation on the magnitude of LTP was also assessed. Results indicated that $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed significant impairments in LTP when compared to WT mice at 2 months. This result is in agreement with other studies which have also demonstrated impaired synaptic plasticity in mouse models of HD, including the 72/ 80 CAG knock-in (Usdin *et al*., 1999), R6/2 (Murphy *et al*., 2000), $Hdh^{Q92}$ and $Hdh^{Q111}$ (Lynch *et al*., 2007) mouse models (see section 7.1). Of specific interest to the mouse model used in the present study, LTP has only previously been assessed in homozygous $Hdh^{Q111}$ mice (Lynch *et al*., 2007). The results of the current study clearly demonstrate that LTP deficits are also evident in the more clinically relevant heterozygous $Hdh^{Q111}$ mice, indicating that similar to the human disorder, only one copy of the mutant gene is necessary to produce the impairments in synaptic plasticity associated with HD.

Specifically, LTP deficits were evident in the 80 CAG mouse model of HD (Usdin *et al*., 1999; see section 7.1). However in 12% of the mutant slices, LTP was indistinguishable from wild type, suggesting that the huntingtin mutation perhaps increases the threshold for LTP induction. In agreement, following an enhanced tetanus stimulus (six 1 second long trains at 100 Hz, 30 seconds apart), LTP in mutant slices was indistinguishable from wild type slices that had been subjected to the same enhanced tetanus stimulation (Usdin *et al.*, 1999). It is perhaps the case that a common mechanism exists between the 80 CAG and the $Hdh^{Q111}$ mouse, so it would be of benefit to determine whether a stronger LTP induction protocol could also relieve the LTP deficit seen in the $Hdh^{Q111}$ mouse.

The study by Lynch *et al.* (2007) observed that LTP was reduced in slices generated from homozygous $Hdh^{Q92}$ and $Hdh^{Q111}$ mice, and in agreement with the present study, did not demonstrate any differences in paired-pulse facilitation, also suggesting a postsynaptic locus of action. Several studies have provided a possible post-synaptic mechanism behind the LTP deficit in the $Hdh^{Q111}$ and these will be discussed in the following section. TBS causes actin polymerization in potentiated synapses, thereby assisting in the stabilisation of LTP (Lin *et al*., 2005; Kramar *et al*., 2006). However, the increase F-actin expression following TBS is absent in $Hdh^{Q111}$ hippocampal slices, perhaps explaining the deficit in the stabilisation of LTP in the $Hdh^{Q111}$ mouse (Lynch *et al*., 2007). In addition, the deficits in actin polymerisation found in the $Hdh^{Q111}$ mouse could also provide an explanation for the abnormal spine morphology found in striatal and cortical neurons in human patients (Graveland *et al*., 1985; Ferrante *et al*., 1991) and mouse models of HD (Guidetti *et al*., 2001; Spires *et al*., 2004). Brain derived neurotrophic factor (BDNF) is a positive modulator of LTP and acts by reducing the

after-hyperpolarisation that results from TBS (Bramham and Messaoud, 2005; Kramar *et al*., 2006) and by facilitating actin polymerisation in spines immediately after TBS (Rex *et al*., 2007). Previous findings have shown that BDNF expression is decreased by the presence of mutant huntingtin (Zuccato *et al.*, 2001, 2005; Spires *et al.*, 2004). In addition, studies have been indicated that BDNF levels are reduced in the neocortex, striatum (Gines *et al*., 2003) and hippocampus (Lynch *et al*., 2007) of mouse models of HD. Lynch *et al.* (2007) demonstrated that the LTP deficit in the $Hdh^{Q111}$ mouse could be rescued by BDNF to a level that was not significantly different from BDNF-treated wild type slices (Lynch *et al*., 2007). This study therefore suggests that the cognitive deficits associated with HD could be treated by increasing BDNF levels, possibly by treatment with an AMPAkine (Lauterborn *et al*., 2000). In agreement, a study by Simmons *et al.* (2009) demonstrated that the LTP and long-term memory deficits in homozygous $Hdh^{Q140}$ mice could be rescued to a level comparable to control following treatment with an AMPAkine (5 mg/kg, injected twice daily for 4 days; Simmons *et al*., 2009). However the study by Lynch *et al.* (2007) only demonstrated reduced BDNF levels and the rescue of LTP by BDNF in $Hdh^{Q111+/+}$ mice. It would therefore be valuable for the BDNF levels to be assessed in the more clinically relevant $Hdh^{Q111+/-}$ mouse to see if reduced BDNF expression is consistent.

In conclusion, I have shown that the $Hdh^{Q111}$ mouse displays deficits in episodic memory and long-term recognition memory (Chapter 6). In addition, this chapter indicates that the $Hdh^{Q111}$ mouse displays LTP deficits (2 months). In the following chapter, data will be presented illustrating that acute treatment of the brain slice preparation with a drug known to enhance certain forms of cognition, can rescue these cognitive and LTP deficits.

# 8: Experiment 4: The cognitive and LTP deficit in $Hdh^{Q111}$ mice is rescued by an $\alpha_5$-$GABA_A$ receptor inverse agonist

## 8.1: Introduction

In the previous chapters I have demonstrated that the $Hdh^{Q111}$ mouse model of HD displays impairments in episodic memory and long-term impairments in the 24 hour novel object recognition task (2 months). In addition, these cognitive deficits are evident before the appearance of an overt motor phenotype in the $Hdh^{Q111}$ mouse. Studies have shown that reduced or deleted expression of $\alpha_5$-$GABA_A$ receptors facilitates cognition (Collinson *et al*., 2002; Crestani *et al*., 2002; Caraiscos *et al*., 2003; Martin *et al*., 2010; see section 4.3). Furthermore, drugs inhibiting the function of $\alpha_5$-$GABA_A$ receptors are known to enhance memory (Chambers *et al*., 2003; Dawson *et al*., 2006; Nutt *et al*., 2007). These studies will be discussed in the introduction section of this chapter. Extrasynaptic $\alpha_5$-$GABA_A$ receptors are densely expressed in mouse hippocampal CA1 neurons (Bai *et al*., 2001; Caraiscos *et al*., 2004; Vargas-Caballero *et al*., 2010). Studies have demonstrated that selective $\alpha_5$-$GABA_A$ receptor antagonists enhance submaximal LTP in this region (Dawson *et al*., 2006; Martin *et al*., 2010). In this chapter I will report the results of experiments examining the effect of $\alpha_5$IA, a selective inverse agonist for the $\alpha_5$-$GABA_A$ receptor (Sternfeld *et al*., 2004) on the cognitive and LTP deficits I have previously demonstrated in the $Hdh^{Q111}$ mouse model of HD.

## 8.1.1: Inverse agonists of the $\alpha_5$-$GABA_A$ receptor enhance cognition and LTP.

The $\alpha_5$-$GABA_A$ receptor is expressed primarily in the hippocampus, suggesting a role in learning and memory (see section 4.3). In addition, pharmacological studies support the role of $\alpha_5$-$GABA_A$ receptors in learning processes. A study by Chambers *et al.* (2003) demonstrated that inverse agonists of the $\alpha_5$-$GABA_A$ receptor enhance cognitive processes in rats. Whilst benzodiazepine agonists such as diazepam increase the GABA-induced chloride flux mediated by $GABA_A$ receptors, non-selective (*i.e.* do not discriminate between $GABA_A$ receptor isoforms) inverse agonists cause a decrease in chloride flux and often an increase in neuronal excitability (Vargas-Caballero *et al*., 2010). Non-selective benzodiazepine inverse agonists enhance cognitive performance in animals (McNamara & Skelton, 1993), but can be anxiogenic (Dorow *et al.*, 1983) and pro-convulsant (Peterson, 1983; Sarter, 2001), thereby restricting the potential use of non-selective inverse agonists as treatments for disorders where memory is impaired. Due to the proposed involvement of the $\alpha_5$-$GABA_A$ receptor in learning and memory Chambers *et al.* (2003) hypothesised that an inverse agonist selective for the $\alpha_5$-$GABA_A$ receptor could be utilised as a cognitive enhancing agent that lacked the unwanted side effects associated with non-selective inverse agonists. Chambers *et al.* (2003) identified a novel inverse agonist, 6,6–Dimethyl–3–(2–hydroxyethyl)thio–1–(thiazol–2–yl)–6,7-dihydro–2–benzothiophen – 4(5H)–one (Compound 43), that had higher affinity at the $\alpha_5$-$GABA_A$ receptor when compared to the other receptor subtypes. Spatial memory was enhanced following treatment with Compound 43 when compared to vehicle-treated animals.

A further selective inverse agonist for the $\alpha_5$-$GABA_A$ receptor was identified in 2004 (Sternfeld *et al*., 2004). The inverse agonist $\alpha_5$IA has a relatively high affinity for human recombinant $GABA_A$ receptors containing the $\alpha_1$, $\alpha_2$, $\alpha_3$ or $\alpha_5$ subunit co-expressed with a $\beta_3$ and a $\gamma$2 subunit (Ki values 0.58 – 0.88 nM), with lower affinity for the equivalent $\alpha_4$ (Ki 60 nM) and $\alpha_6$ (Ki 418 nM) subunit containing receptors. A similar affinity of $\alpha_5$IA for native rat $GABA_A$ receptors (Ki ~1nM) was determined, suggesting that $\alpha_5$IA does not show interspecies differences. A study by Dawson *et al.* (2006) demonstrated that $\alpha_5$IA improved performance in a hippocampal-dependent version of the MWM task. The anxiolytic properties of $\alpha_5$IA were tested by assessing the performance of the rats in the elevated plus maze. The elevated plus maze has previously been shown to be sensitive to agonists and inverse agonists acting at the benzodiazepine binding site of the $GABA_A$ receptor (Pellow and File, 1986). Mice were treated with a non-selective partial inverse agonist at the benzodiazepine binding site (FG 7142), the $\alpha_5$-selective inverse agonist $\alpha_5$IA, or were treated with vehicle. FG 7142 significantly increased the amount of time spent in the closed arms of the elevated plus maze, suggesting it had an anxiogenic-like effect. In contrast, $\alpha_5$IA-treated rats did not show any differences in the time spent in the closed arms. The sedative properties of $\alpha_5$IA were also assessed. The effect of $\alpha_5$IA and diazepam on the locomotor activity of mice was demonstrated using a fixed speed rotarod protocol. Diazepam reduced the latency to fall from the rod in a dose-dependent manner, whereas $\alpha_5$IA had no effect (Dawson *et al*., 2006). Finally the pro-convulsant activity of $\alpha_5$IA was assessed. Mice were either injected with vehicle, $\alpha_5$IA or FG 7142 and were subsequently infused with the pro-convulsant pentylentetrazole and the time taken to induce clonic and full tonic seizures was measured. Administration of $\alpha_5$IA did not induce seizures, nor did it decrease the dose of pentylentetrazole required to induce convulsions. By contrast, FG

7142 reduced the dose of pentylenetetrazole required to induce a seizure, indicating pro-convulsant activity. This study therefore shows that selective inhibition of $\alpha_5$-$GABA_A$ receptors resulted in improved performance in the hippocampal-dependent MWM task, without the anxiogenic, sedative or convulsant properties associated with non-selective $GABA_A$ receptor inverse agonists (Dawson *et al*., 2006).

In addition to studying the *in vivo* effects of $\alpha_5$IA, Dawson *et al.* (2006) also investigated the *in vitro* properties of this inverse agonist. LTP in the Schaffer collateral-commissural pathway of the hippocampus was induced by a brief tetanus (10 stimuli at 100 Hz) followed by a TBS and the effect of $\alpha_5$IA on the magnitude of LTP was assessed. Dawson *et al.* (2006) found that although $\alpha_5$IA had no effect on basal synaptic transmission, LTP was significantly enhanced in $\alpha_5$IA-treated slices when compared to controls. As previous studies have indicated that non-selective inverse agonists at the benzodiazepine binding site potentiate LTP (Seabrook *et al.*, 1997), enhancement of LTP following disinhibition *via* the $\alpha_5$-$GABA_A$ receptor using $\alpha_5$-subunit selective inverse agonists indicates that the $\alpha_5$-subtype is potentially responsible for the LTP enhancement observed with non-selective inverse agonists.

A study by Nutt *et al.* (2007) used $\alpha_5$IA to investigate the role of $\alpha_5$-$GABA_A$ receptors in cognition in man. In addition to being modulated by benzodiazepines, there is some evidence, albeit controversial, that $GABA_A$ receptors are also modulated by alcohol (Wallner *et al*., 2003; 2006; Olsen *et al*., 2007), however see Borghese *et al*., 2006. Alcohol has been shown in some studies to influence the function of the $GABA_A$ receptor by enhancing the effects of GABA and other agonists that act on certain $GABA_A$ receptor isoforms. The $\alpha_5$-$GABA_A$ receptor has been implicated in mediating

the effects of alcohol including motor co-ordination, sedation and amnesia (McKay *et al.*, 2004; Pickering *et al.*, 2007). Taking this into consideration, the study by Nutt *et al.* (2007) used $\alpha_5$IA to evaluate the role of the $\alpha_5$-$GABA_A$ receptor in mediating the effects of alcohol on the brain. In this study volunteers were dosed with either $\alpha_5$IA, or placebo and were given alcohol to drink. One hour after they had consumed the alcohol the subjects were asked to memorise a list of 20 words and recall them 30 minutes later. The study determined that the adverse memory effects of alcohol were attenuated by $\alpha_5$IA. When subjects were pre-treated with $\alpha_5$IA they were able to recall significantly more words than when they were treated with vehicle. In addition, subjects who had consumed more alcohol showed a more significant improvement in memory recall after treatment with $\alpha_5$IA. Other than the enhancement of memory, $\alpha_5$IA had no significant effect on the other functional impairments associated with alcohol consumption, including motor co-ordination and sedation. In agreement with previous studies in rodents (Chambers *et al.*, 2003, Dawson *et al.*, 2006), this study demonstrated that the $\alpha_5$-$GABA_A$ receptor in humans is important in the regulation of learning and memory, indicating that this receptor could potentially be a therapeutic target in the early stages of diseases that are associated with cognitive decline.

### 8.1.2: Specific targeting of the $\alpha_5$-$GABA_A$ receptor in an animal model of cognitive decline.

Down's syndrome is a disorder that is characterised by varying degrees of cognitive decline and an imbalance between inhibitory and excitatory neurotransmission, specifically increased GABAergic transmission, is thought to contribute to the learning and memory abnormalities associated with individuals with the disorder (Best *et al.*, 2007; Kleschevnikov *et al.*, 2004). In agreement, treatment of mouse models of Down's

syndrome with the non-competitive $GABA_A$ receptor antagonists picrotoxin and pentylenetetrazol have been demonstrated to restore cognitive decline (Fernandez *et al.*, 2007; Rueda *et al.*, 2008). However, these drugs are convulsant at high doses. Subsequently a recent study by Braudeau *et al.* (2011) assessed the effects of $\alpha_5$IA in a cognitively impaired Ts65Dn mouse model of Down's syndrome. Braudeau *et al.* (2011) showed that $\alpha_5$IA treatment rescued the spatial deficits associated with Ts65Dn mice. In addition, Ts65Dn mice showed an impaired performance in the novel object recognition task that could be recovered following pre-treatment with $\alpha_5$IA. Due to the lack of convulsant or anxiogenic effects in the Ts65Dn mouse, $\alpha_5$IA demonstrates a more favourable therapeutic profile than non-selective GABAergic drugs. This study therefore provides evidence for the potential use of selective inverse agonists at the $\alpha_5$-$GABA_A$ receptor for the treatment of diseases associated with cognitive decline (Braudeau *et al*., 2011).

## 8.2: Methods

The role of $\alpha_5$-$GABA_A$ receptors in the regulation of learning and memory indicates that this receptor could potentially be a therapeutic target in diseases of cognitive decline. The effects of $\alpha_5$IA (Sternfeld *et al*., 2004) on the cognitive and the LTP deficits in the $Hdh^{Q111}$ mouse model of HD will be discussed in the following chapter.

### 8.2.1: Drug application – Cognition tests

Following training in the novel object, object-place and object-context tasks, WT ($n$ = 12) and $Hdh^{Q111+/-}$ ($n$ = 12) were tested in the object-place-context task (section 6.2.1). On each day mice received intraperitoneal injections of either $\alpha_5$IA (3 mg / kg), vehicle (0.4% methylcellulose) or were not injected. For the long protocol (section 6.2.1) each

mouse performed the task 12 times, receiving 4 drug and 4 vehicle injections and 4 trials with no injection. For the short protocol (section 6.2.2), each mouse performed the task 3 times, receiving 1 drug and 1 vehicle injection and 1 trial with no injection. In the 24 hour novel object recognition task (section 6.2.3), mice were only injected on the sample days (*i.e.* mice were not injected on the test day). Each mouse performed the task 3 times, one for each condition, and therefore each mouse received 2 drug and 2 vehicle injections (and 1 trial with no injection). In all tests, injections were counterbalanced to eliminate any object bias that may occur on a given day within the experimental groups and the experimenter was blind to the identity of the injected solutions. After injection, the mouse was placed back into the home cage and was tested 30 minutes later.

### 8.2.2: Drug application – LTP

Hippocampal slices from WT and $Hdh^{Q111+/-}$ mice were perfused with $\alpha_5$IA (300 nM) for at least 30 minutes prior to the induction of LTP (for electrophysiological methods, see section 7.2).

## 8.3: Results

### 8.3.1: Episodic memory (long protocol) $\alpha_5$IA drug treatment

The episodic memory of WT ($n$ = 12) and $Hdh^{Q111+/-}$ ($n$ = 12) mice was assessed (2 months). A repeated measures ANOVA was performed on discrimination scores with genotype (WT *vs.* $Hdh^{Q111+/-}$) as the between subjects factor and drug condition (no injection *vs.* vehicle *vs.* $\alpha_5$IA) as the within subjects factor. This showed a significant effect of genotype ($f_{(1, 22)} = 93.827$, $P < 0.05$), drug condition ($f_{(2, 44)} = 3.680$, $P < 0.05$)

and a significant drug condition *vs*. genotype interaction ($f_{(2, 44)} = 9.037$, $P < 0.05$) (2 months). Bonferroni corrected pair-wise comparisons confirmed that $Hdh^{Q111+/-}$ mice showed impaired performance in the object-place-context task when compared to WT irrespective of whether they received no injection, or whether they were injected with vehicle (both $P < 0.05$; Figure 8.1A and 8.1B). Furthermore, a one-sample *t*-test indicated that WT ($P < 0.05$), but not $Hdh^{Q111+/-}$ ($P > 0.05$) mice, explored the novel configuration significantly more than expected by chance in these two conditions, *i.e.* $Hdh^{Q111+/-}$ mice were unable to identify the novel object regardless of whether they were injected with vehicle, or did not receive an injection. Bonferroni corrected pair-wise comparisons confirmed that, following injection with 3 mg/ kg $\alpha_5$IA, the performance of the compromised $Hdh^{Q111+/-}$ mice was improved to a level that was not significantly different from $\alpha_5$IA-treated WT mice ($P > 0.05$; Figure 8.1C). Finally, one-sample *t*-tests confirmed that both WT and $Hdh^{Q111+/-}$ mice explored the novel configuration significantly more than expected by chance following injection with $\alpha_5$IA.

In addition, in order to demonstrate that $\alpha_5$IA did not remain in the system over night and influence the performance of the mice the next day, analysis was carried out comparing the discrimination indices of WT mice that received an $\alpha_5$IA injection followed by a vehicle injection the next day and *vice versa*. The data was arranged in categories according to the order of drug injections from day to day *i.e.* vehicle following drug or drug following vehicle. A one-way ANOVA demonstrated that the order of the injections does not affect the discrimination indices obtained ($f_{(1, 36)} = 0.750$, $P > 0.05$), indicating that $\alpha_5$IA does not remain in the system over night. In addition, pharmacokinetic studies in man have also shown that the half-life of $\alpha_5$IA is

approximately 2 – 2.5 hours (Xue *et al*., 2004), therefore providing further evidence that the order of injections should not influence the results.

The exploration times of mice in each condition were assessed. One way ANOVAs showed no significant differences in the exploration patterns of WT and $Hdh^{Q111+/-}$ mice in the sample and test phases in the 3 drug conditions (all $P > 0.05$) (Figure 8.1A and 8.1B).

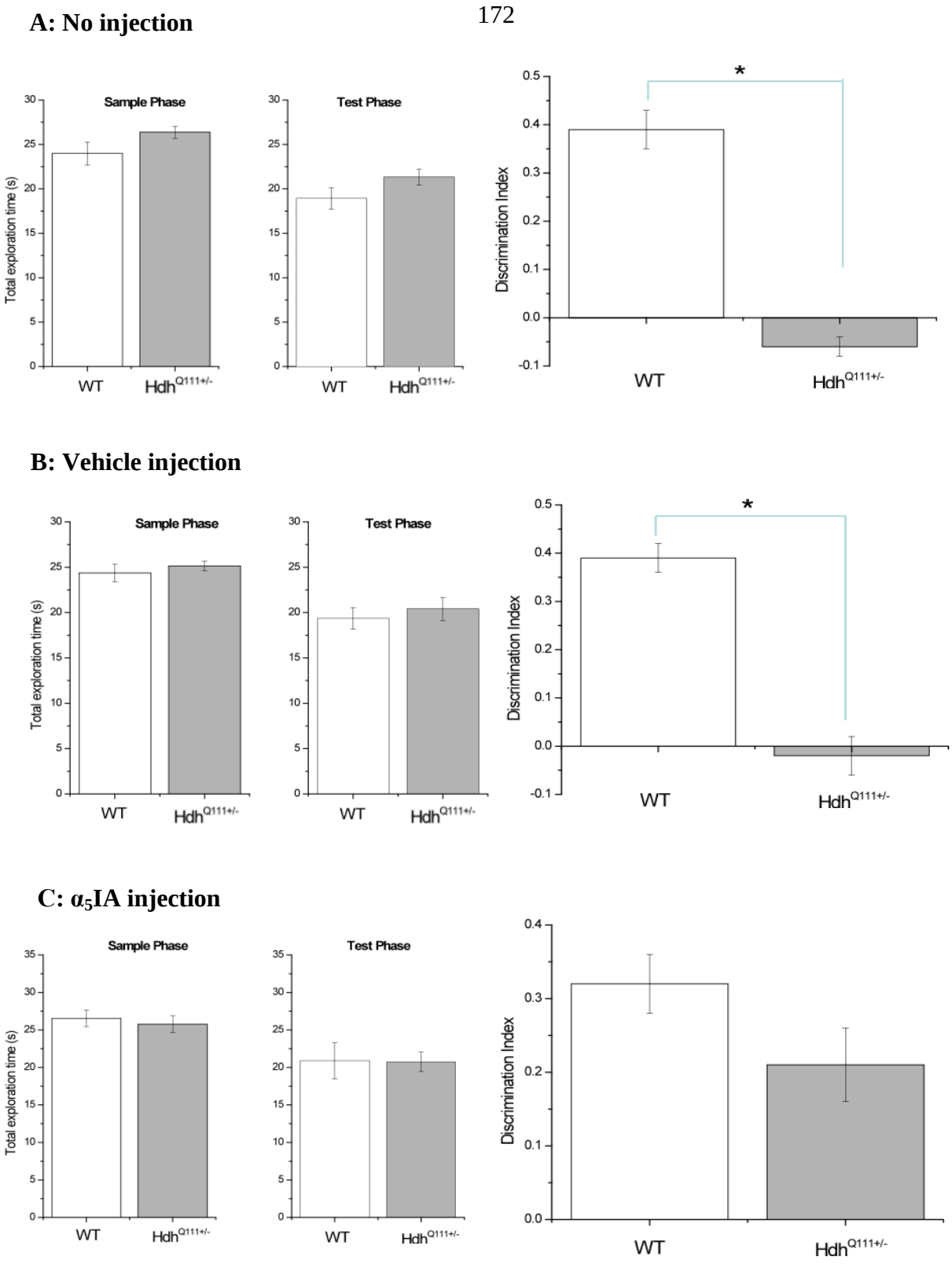



***Figure 8.1: $\alpha_5$IA greatly improves the cognitive deficit of Hdh$^{Q111+/-}$ mice in the episodic memory task.*** *Graphs show the exploration times (left) and discrimination indices (right). Hdh$^{Q111}$ mice (n = 12) are significantly impaired when compared to WT in their ability to identify the novel object in the no injection (A), and vehicle injection trials (B) ($P < 0.05$). (C) The performance of Hdh$^{Q111+/-}$ mice is significantly improved following injection with $\alpha_5$1A, ($P < 0.05$) to a level that is not significantly different from $\alpha_5$1A-treated WT mice ($P > 0.05$). All mice show similar exploration patterns in each of the drug conditions ($P > 0.05$).*

## 8.3.2: Episodic memory (short protocol) $\alpha_5$IA drug treatment

In addition, WT (*n* = 9) and $Hdh^{Q111+/-}$ (*n* = 8) mice were tested in the object-place-context task in order to determine if the rescue of the cognitive impairment of the $Hdh^{Q111+/-}$ with $\alpha_5$IA can still be seen using the short protocol.

The discrimination indices for the three drug conditions were assessed. A repeated measures ANOVA was performed on discrimination scores with genotype (WT *vs*. $Hdh^{Q111+/-}$) as the between subjects factor and drug condition (no injection *vs*. vehicle *vs*. $\alpha_5$IA) as the within subjects factor. This investigation showed a significant effect of genotype ($f_{(1, 15)} = 35.426$, $P < 0.05$), but no effect of the drug condition ($f_{(2, 30)} = 1.794$, $P > 0.05$) or a drug condition *vs*. genotype interaction ($f_{(2, 30)} = 1.890$, $P < 0.05$; 2 months). As a result, no further statistical analysis could be performed. However, the pattern of results using this shortened protocol (Figure 8.2) was similar to that obtained in the longer protocol (Figure 8.1) with $Hdh^{Q111+/-}$ mice showing a trend towards impaired performance in the no injection and vehicle injection trials but improved performance following treatment with $\alpha_5$IA (Figure 8.2C).

The exploration times of the mice in each condition were assessed. One way ANOVAs showed no significant differences in the exploration patterns for sample and test phases in all 3 conditions (all $P > 0.05$) (Figure 8.2).

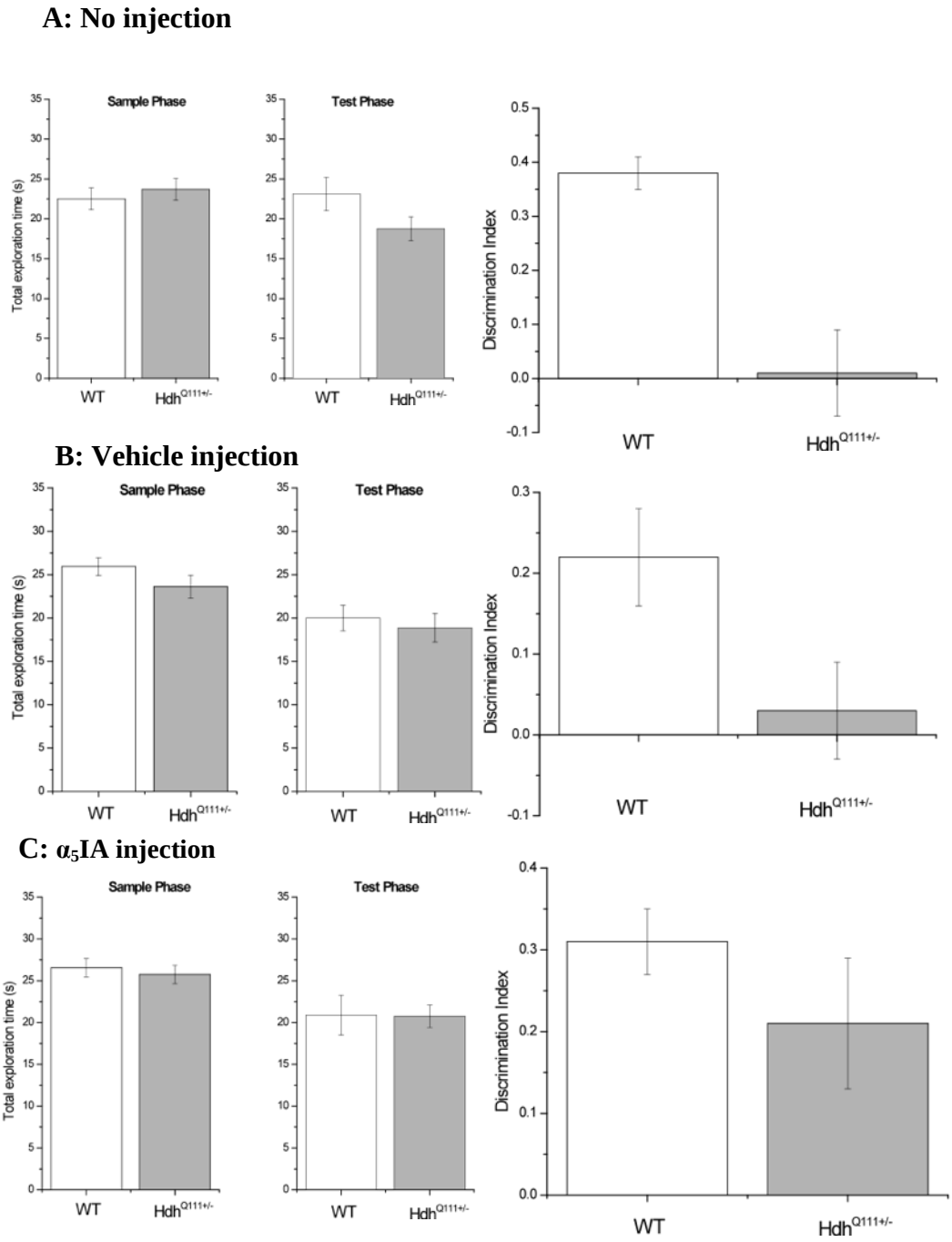



***Figure 8.2**: **$\alpha_5$IA greatly improves the cognitive deficit of Hdh$^{Q111+/-}$ mice in the shortened version of the episodic memory task.** Graphs show the exploration times (left) and discrimination indices (right). A repeated measures ANOVA showed no significant drug vs. genotype interaction, therefore no further statistical analysis could be performed. However, the pattern of results is similar to that obtained using the long protocol in as far as Hdh$^{Q111+/-}$ (n = 8) mice showed impaired performance in the object-place-context task when compared to WT (n = 9) following no injection (A) and vehicle injection (B). (C) The performance of Hdh$^{Q111+/-}$ mice is improved following injection with $\alpha_5$1A, to a level that is similar to $\alpha_5$1A-treated WT mice. All mice show similar exploration patterns in each of the drug conditions (one-way ANOVA, P > 0.05)*

## 8.3.3: 24 hour novel object recognition task : $\alpha_5$IA drug treatment

WT (*n* = 9), $Hdh^{Q111+/+}$ (*n* = 5) and $Hdh^{Q111+/-}$ (*n* = 9) mice were tested in the 24 hour novel object recognition task (Figure 8.3). A repeated measures ANOVA was performed on discrimination scores with genotype (WT *vs.* $Hdh^{Q111+/+}$ *vs*. $Hdh^{Q111+/-}$) as the between subjects factor and drug condition (no injection *vs*. vehicle *vs*. $\alpha_5$IA) as the within subjects factor. Results revealed a significant effect of genotype ($f_{(2, 20)} = 30.666$, $P < 0.05$), drug condition ($f_{(2, 40)} = 13.0639$, $P < 0.05$) and a genotype *vs.* drug condition interaction ($f_{(4, 40)} = 7.492$, $P < 0.05$). Bonferroni corrected pair-wise comparisons between groups confirmed that the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were impaired when compared to WT mice irrespective of whether they received no injection or were injected with vehicle (both $P < 0.05$). Furthermore, one-sample *t*-tests indicated that WT ($P < 0.05$), but not the $Hdh^{Q111+/+}$ or $Hdh^{Q111+/-}$ ($P > 0.05$) mice, performed significantly better than expected by chance in these two conditions, *i.e.* the $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice were unable to identify the novel object after a 24 hour retention interval. However, following injection with 3 mg/ kg $\alpha_5$IA, performance of the compromised $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice was significantly improved ($P < 0.05$) to a level that was not significantly different to their WT counterparts ($P > 0.05$). One-sample *t*-tests indicated that WT, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice, performed significantly better than expected by chance following injection with $\alpha_5$IA.

The exploration patterns of the mice were also assessed. In the no injection trial, one way ANOVAs demonstrated exploration was similar in sample 1 ($f_{(2, 22)} = 2.981$, $P > 0.05$) and sample 2 ($f_{(2, 22)} = 1.997$, $P > 0.05$), but differed in the test phase ($f_{(2, 22)} = 8.727$, $P < 0.05$). Bonferroni corrected pair-wise comparisons confirmed the reduced exploration of the $Hdh^{Q111+/+}$ mice in the test phase ($P < 0.05$) and showed that the

activity of WT and $Hdh^{Q111+/-}$ mice was similar in all three phases ($P > 0.05$). $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed similar exploration patterns in the vehicle trial ($P > 0.05$) (Figure 8.3A).

The exploration patterns for the vehicle trial were analysed. One way ANOVAs demonstrated that exploration was similar in sample 1 ($f_{(2, 22)} = 0.901$, $P > 0.45$) and sample 2 ($f_{(2, 22)} = 2.219$, $P > 0.05$), but differed in the test phase ($f_{(2, 22)} = 5.845$, $P < 0.05$). Bonferroni corrected pair-wise comparisons confirmed the reduced exploration of the $Hdh^{Q111+/+}$ mice in the test phase ($P < 0.05$) and showed that the activity of WT and $Hdh^{Q111+/-}$ mice was similar in all three phases ($P > 0.05$). $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed similar exploration patterns in the vehicle trial ($P > 0.05$) (Figure 8.3B).

Finally the exploration patterns for the $\alpha_5$IA trial were assessed. One-way ANOVAs showed significant differences in sample 1 ($f_{(2, 22)} = 3.908$, $P < 0.05$) and sample 2 ($f_{(2, 22)} = 4.994$, $P < 0.05$). Bonferroni corrected pair-wise comparisons between groups for each task showed that $Hdh^{Q111+/-}$ mice explored significantly less than WT mice in both the sample phases ($P < 0.05$), whereas $Hdh^{Q111+/+}$ mice explored similarly to WT ($P > 0.05$). However, although $Hdh^{Q111+/-}$ mice displayed hypoactivity in the sample phase, episodic memory was still enhanced following injection with $\alpha_5$IA. Exploration was similar in the test phase ($f_{(2, 22)} = 0.496$, $P > 0.05$) (Figure 8.3C).

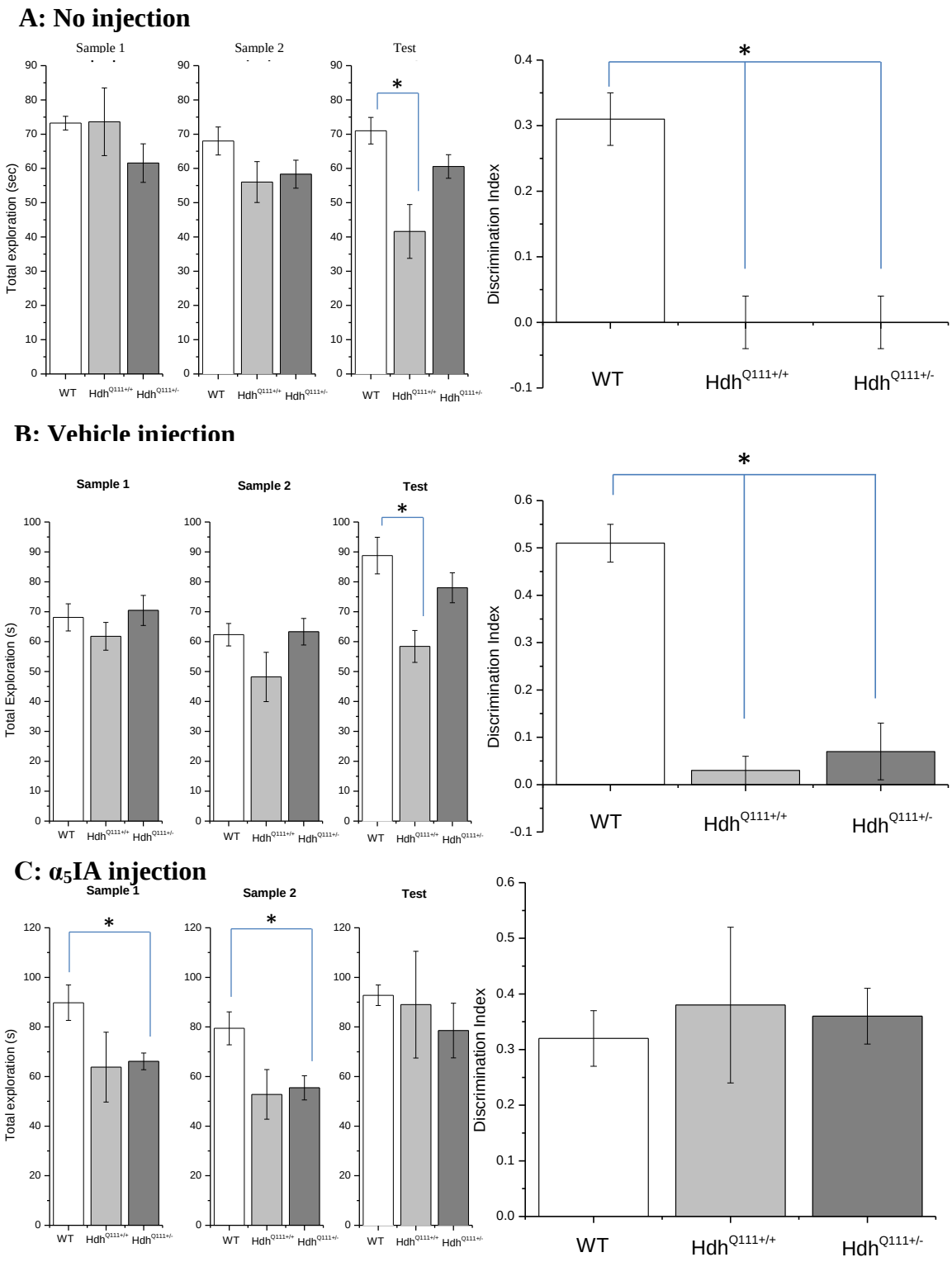



***Figure 8.3: $\alpha_5$IA greatly improves the cognitive deficit of $Hdh^{Q111+/-}$ mice in the 24 hour novel object recognition task.***

*For each panel: Left shows the total exploration times of the WT (n = 9), $Hdh^{Q111+/+}$ (n = 5) and $Hdh^{Q111+/-}$ (n = 9) mice in Sample 1, Sample 2 and test phase. Right shows the discrimination index in the test phase. $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice are significantly impaired in their ability to identify the novel object when compared to WT in the (A) no injection and (B) vehicle injection trials* ($P < 0.05$). (C) *The performance of $Hdh^{Q111+/+}$and $Hdh^{Q111+/-}$ mice is improved following injection with $\alpha_5$1A, to a level that is similar to $\alpha_5$IA-treated WT mice ($P > 0.05$).*

## 8.3.4: The effects of the $\alpha_5$-$GABA_A$ receptor inverse agonist $\alpha_5$IA on hippocampal LTP of WT and $Hdh^{Q111}$ mice.

Previous studies have demonstrated that selective $\alpha_5$-$GABA_A$ receptor antagonists enhance submaximal hippocampal LTP (Dawson *et al*., 2006; Martin *et al*., 2010; section 8.1.1). Given the association of LTP and memory (section 2.3) and the fact that $\alpha_5$IA rescued the cognitive abnormalities of $Hdh^{Q111}$ mice (sections 8.3.1 – 8.3.3), the effect of $\alpha_5$IA on the LTP deficit associated with $Hdh^{Q111}$ mice (section 7.3.3) was assessed.

### 8.3.4.1: The influence of the stimulus protocol on the magnitude of LTP.

For hippocampal slices derived from WT mice ($n$ = 14), a 4-TBS induced a robust form of LTP (93 ± 16 % increase in the slope of the fEPSP), as measured 50 minutes post-TBS (2 months) (Figure 8.4). LTP was also induced using the sub-maximal 3-TBS (Figure 8.4). In WT slices the 3-TBS induced LTP, but of a magnitude (32 ± 4 % increase, $n$ = 6) significantly less than that induced by the 4-TBS paradigm (repeated measures ANOVA, $f_{(1, 20)} = 8.486$, $P < 0.05$).

### 8.3.4.2: The magnitude of LTP following a sub-threshold stimulus is enhanced by an $\alpha_5$-$GABA_A$ receptor "inverse agonist".

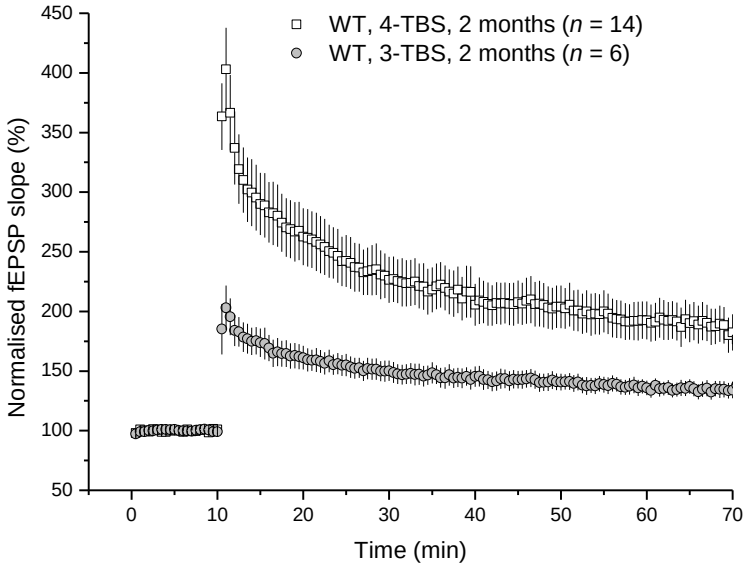
As a prelude to investigating whether the $\alpha_5$-$GABA_A$R inverse agonist $\alpha_5$IA (Sternfeld *et al*., 2004) may rescue the deficit in LTP noted in $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice (see section 7.3.3), I investigated the effect of $\alpha_5$IA on the submaximal LTP induced in WT hippocampal slices by a 3-TBS ($n$ = 8). WT hippocampal slices were perfused with $\alpha_5$IA (300 nM) for at least 30 minutes prior to the induction of LTP. This treatment did

not change baseline (*i.e.* control fEPSPs) transmission in WT slices. However, in the continued presence of 300 nM $\alpha_5$IA, LTP (obtained 50 minutes after 3-TBS) is significantly greater (86 ± 22 % increase; $n$ = 8) than that obtained from untreated WT slices following 3-TBS (36 ± 6 % increase; $n$ = 6; repeated measures ANOVA, $f_{(2, 25)}$ = 4.338, $P < 0.05$). Furthermore, Bonferroni corrected pair-wise comparisons between groups confirmed that, in $\alpha_5$IA-treated WT slices, LTP following 3-TBS was not significantly different from the robust LTP following 4-TBS in untreated WT slices ($P > 0.05$; Figure 8.5).

Additionally, the effect of 300 nM $\alpha_5$IA on LTP following 4-TBS was also assessed. In $\alpha_5$IA-treated WT brain slices ($n$ = 10), the magnitude of LTP (obtained 50 minutes after 4-TBS) was a 64 ± 8 % increase of the fEPSP slope *c.f.* control, a value that was not significantly different from the magnitude of LTP induced by the 4-TBS protocol in untreated WT brain slices (93 ± 19 % increase, repeated measures ANOVA, $f_{(1, 22)}$ = 12.898; $P > 0.05$; Figure 8.6).

In summary, these data illustrates that the magnitude of LTP resulting from a sub-threshold 3-TBS in WT slices can be facilitated by the $\alpha_5$-$GABA_A$ receptor inverse agonist $\alpha_5$IA. However, $\alpha_5$IA has no effect on the robust LTP following 4-TBS in WT slices.

**A**




**B**

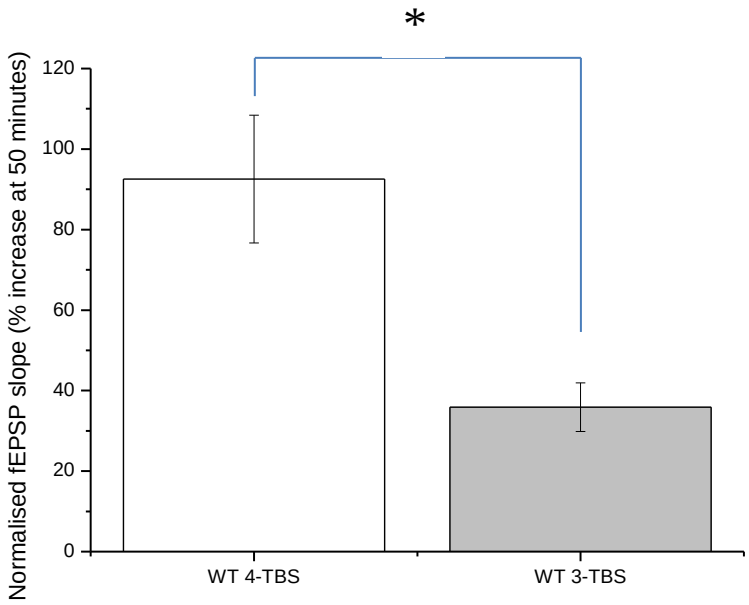



***Figure 8.4: A comparison of LTP induced by a 4-TBS and a 3-TBS protocol in hippocampal slices derived from 2 month old WT mice.***

*A) The time course of the changes in the slope of the fEPSP after the induction of LTP by a 4-TBS or 3-TBS, delivered after the establishment of a 10 minute control baseline. The fEPSP slope is plotted as a percentage change against time and expressed as a pooled mean (± SEM). B) A 4-TBS induced a robust LTP (93 ± 16% increase; determined 50 mins. post-TBS) in WT hippocampal slices. The magnitude of LTP was significantly less following a 3-TBS (36 ± 6% increase, $P < 0.05$) cf. the 4-TBS. Error bars indicate SEM. n values indicates the number of brain slices.*

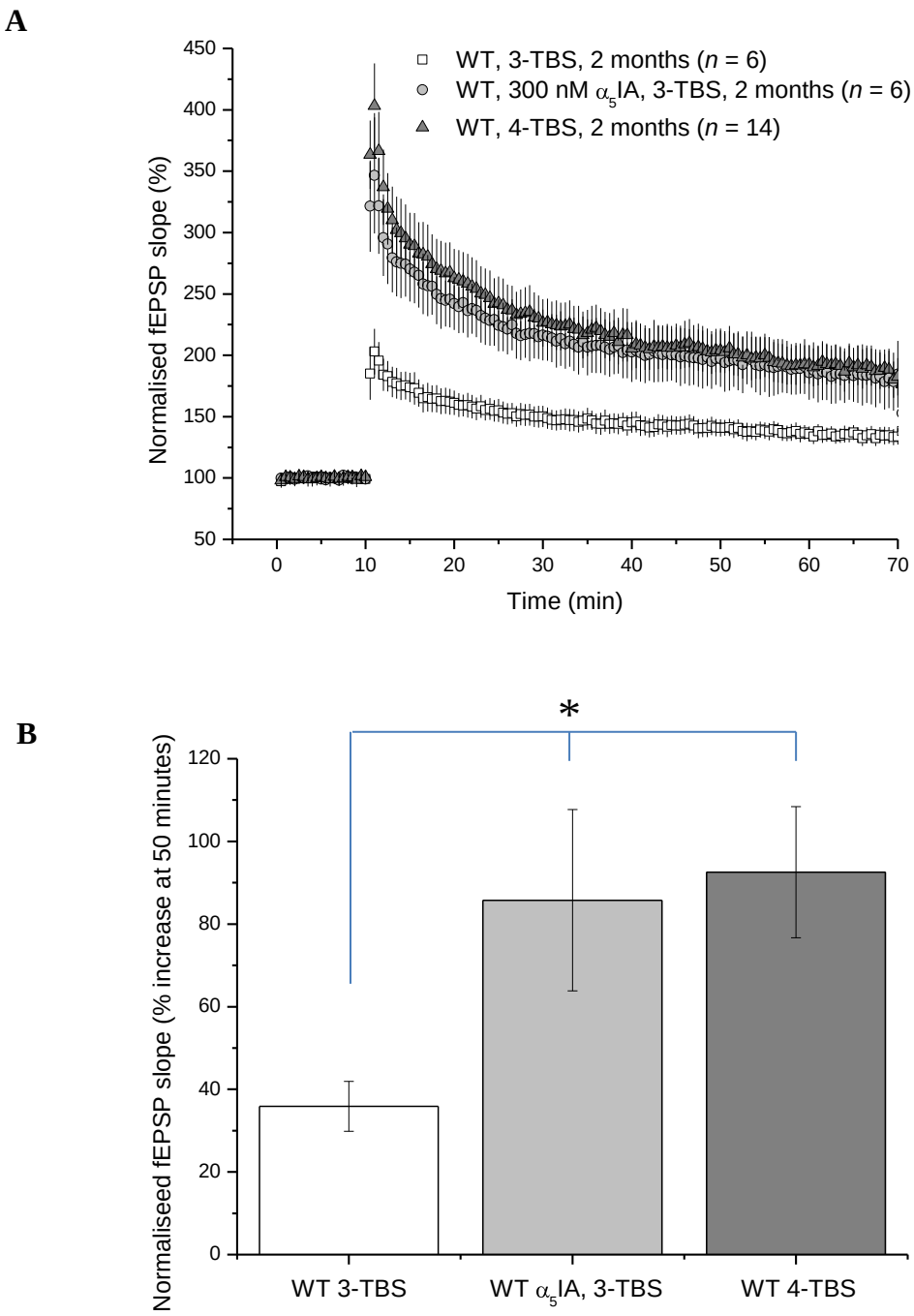
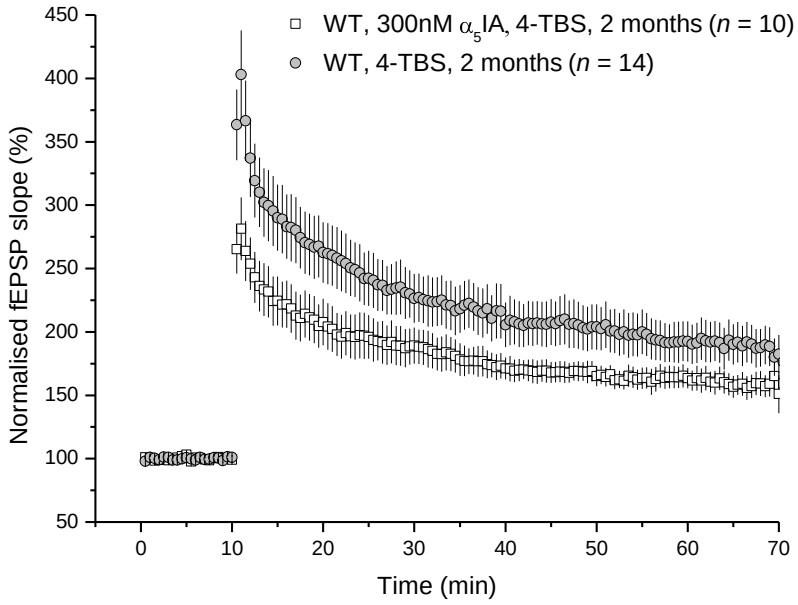



***Figure 8.5: In WT hippocampal brain slices sub-maximal LTP is facilitated by $\alpha_5$IA***

*A) The time course of the changes to the slope of the fEPSP after the induction of LTP induced by a 3-TBS, delivered after the establishment of a 10 minute control baseline. The fEPSP slope is plotted as a percentage change against time and expressed as a pooled mean (± SEM). B) The magnitude of LTP was significantly increased following 3-TBS in WT slices treated with 300 nM $\alpha_5$IA (86 ± 22% increase; n = 8) when compared to control 3-TBS LTP (*36 ± 6% *increase; n = 6) (P < 0.05). Note the magnitude of LTP following a 3-TBS in brain slices treated with $\alpha_5$IA is not significantly different from that induced by a 4-TBS in untreated WT slices (P > 0.05; n = 14). Error bars indicate SEM. n values indicate number of slices.*

**A**




**B**

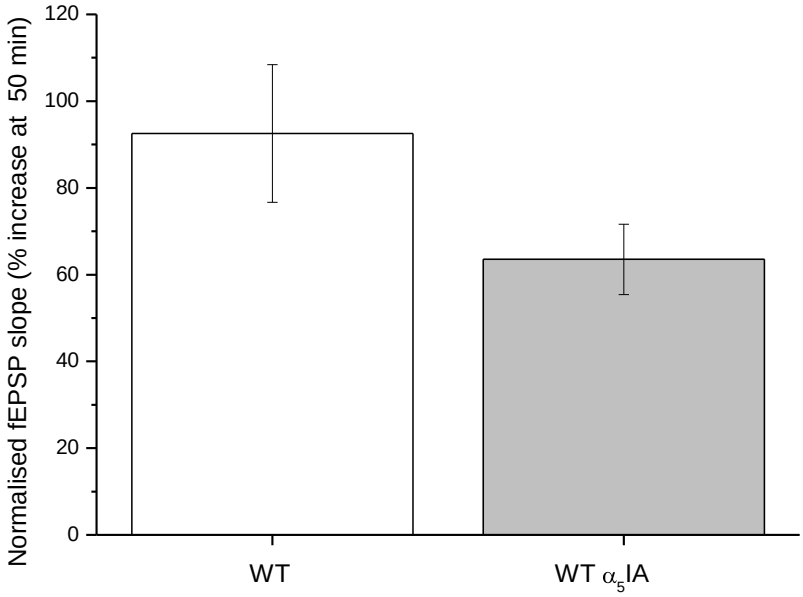



***Figure 8.6: The $\alpha_5$-GABA$_A$R inverse agonist $\alpha_5$IA has no effect on LTP induced by 4-TBS in hippocampal slices derived from WT (2month old) mice.***

*A) The time course of the changes in the slope of the fEPSP after the induction of LTP by a 4-TBS, applied after the establishment of a 10 minute control baseline. The fEPSP slope is plotted as the percentage change against time and expressed as a pooled mean (± SEM). B) The magnitude of LTP was unchanged in brain slices treated with 300 nM $\alpha_5$IA (64 ± 8% increase; n = 10) when compared to control 4-TBS LTP (93 ± 16% increase; n = 14; $P < 0.05$). Error bars indicate SEM. n values indicate number of brain slices.*

**8.3.4.3: The impairment of LTP in 2 month old $Hdh^{Q111}$ mice is rescued by an $\alpha_5$-$GABA_A$ receptor inverse agonist.**

The following section will report the effect of $\alpha_5$IA on the LTP deficits observed in $Hdh^{Q111+/-}$ brain slices (2 months old; see section 7.3.3).

In $Hdh^{Q111+/-}$ brain slices that had been treated with 300 nM $\alpha_5$IA ($n$ = 12), the magnitude of LTP following a 4-TBS was significantly greater (62 ± 11 % increase) than that of untreated $Hdh^{Q111+/-}$ brain slices (28 ± 6 % increase; $n$ = 15; repeated measures ANOVA, $f_{(1, 23)} = 7.067$, $P < 0.05$; Figure 8.7). Furthermore, potentiation of the fEPSP slope 50 minutes after 4-TBS in $Hdh^{Q111+/-}$ brain slices treated with 300 nM $\alpha_5$IA was not significantly different to that seen in WT brain slices that had been treated with 300 nM $\alpha_5$IA (64 ± 8 % increase; $n$ = 10; repeated measures ANOVA, $f_{(1, 20)} = 0.253$, $P > 0.05$; Figure 8.7). These results indicate that treatment of $Hdh^{Q111+/-}$ brain slices with 300 nM $\alpha_5$IA significantly rescues the LTP deficit observed for untreated $Hdh^{Q111+/-}$ brain slices, to a level that is not significantly different from that of WT brain slices that have been treated with 300 nM $\alpha_5$IA.

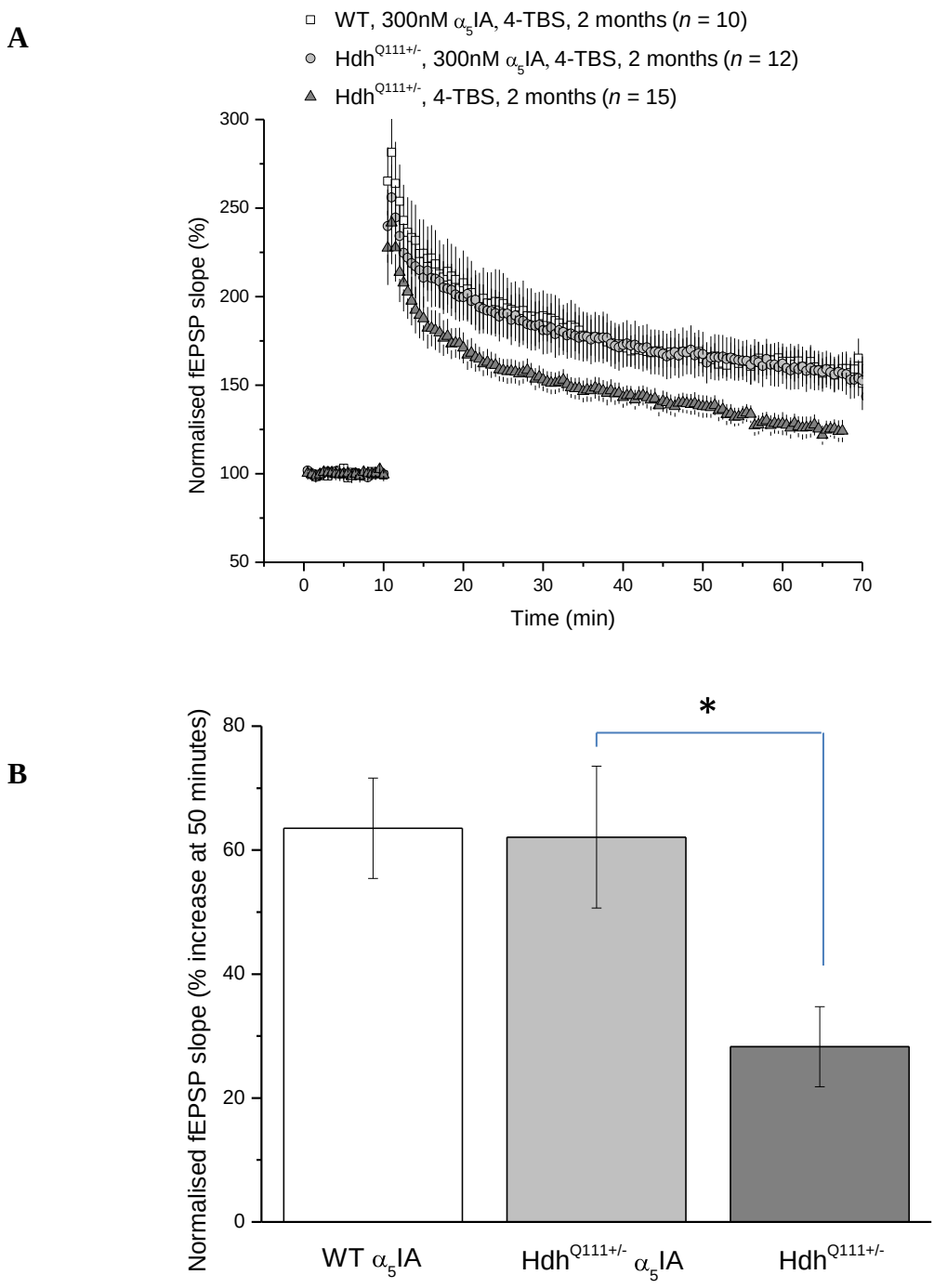



***Figure 8.7: $\alpha_5$IA rescues the deficit of LTP evident in Hdh$^{Q111+/-}$ hippocampal slices***

*A) The time course of the changes in the slope of the fEPSP after the induction of LTP by a 4-TBS, applied after the establishment of a 10 minute control baseline. The fEPSP slope is plotted as the percentage change against time and expressed as a pooled mean (± SEM). B) The magnitude of LTP in Hdh$^{Q111+/-}$ slices treated with 300 nM $\alpha_5$IA (62 ± 11% increase; n = 12) was not significantly different to WT slices treated with 300 nM $\alpha_5$IA (64 ± 8% increase; n = 10; P < 0.05). The magnitude of LTP induced by a 4-TBS in Hdh$^{Q111+/-}$ slices treated with 300 nM $\alpha_5$IA (62 ± 11% increase; n = 12) was significantly greater than that induced in untreated Hdh$^{Q111+/-}$ slices (28 ± 6% increase; n = 15; P < 0.05). Error bars indicate SEM. n values indicate number of brain slices.*

## 8.4: Discussion

In this chapter I have demonstrated that $\alpha_5$IA reverses the deficits in episodic memory in $Hdh^{Q111}$ mice (long-protocol) to a level that is not significantly different from WT mice. Although the pattern of results was similar in the shortened protocol, the cognitive deficit rescue by $\alpha_5$IA was not statistically significant. This was perhaps due to increased variability in the data associated with the shortened protocol. This observation suggests that although this novel shortened protocol is useful for the assessment of cognitive deficits when the developmental window is relatively brief (see sections 6.5 and 6.7), this paradigm is not necessarily a replacement for the longer protocol, where repeated measurements reduce the error in the data set.

In addition I have demonstrated that $\alpha_5$IA alleviates the cognitive deficits of $Hdh^{Q111}$ mice in the 24-hour novel object recognition task to a level that is not significantly different from $\alpha_5$IA-treated WT mice. The rescue of the long-term memory deficit in the $Hdh^{Q111+/-}$ mice is in contrast to a previous study in which the performance of a mouse model of Down's Syndrome in a short-term, but not a long-term, memory task was improved following treatment with $\alpha_5$IA (Braudeau *et al*., 2011; section 8.1.2). Although previous studies have assessed the effect of selective $\alpha_5$-$GABA_A$ receptor inverse agonists on the acquisition and retrieval of stored information, they used memory paradigms that were based on short-term memory retentions that did not assess long-term (at least 24 hours) recall (Atack *et al*., 2006; Collinson *et al*., 2006). However, the current study clearly demonstrates that $\alpha_5$IA has the capacity to improve both short- and long-term memory in cognitively impaired mice.

Furthermore, studies have demonstrated that selective $\alpha_5$-$GABA_A$ receptor antagonists enhance submaximal LTP (Dawson *et al*., 2006; Martin *et al*., 2010). In agreement, I have demonstrated that while $\alpha_5$IA has no significant effect on the magnitude of control LTP, treatment of $Hdh^{Q111+/-}$ brain slices with 300 nM $\alpha_5$IA rescues the LTP deficit observed for untreated $Hdh^{Q111+/-}$ brain slices, to a level that is not significantly different from that of $\alpha_5$IA-treated WT brain slices.

The rescue of the cognitive and LTP deficits in $Hdh^{Q111}$ mice by a selective inverse agonist for the $\alpha_5$-$GABA_A$ receptor suggests that the phenotype may perhaps result from an imbalance between excitatory and inhibitory neurotransmission. Due to the fact that HD is associated with the major loss of striatal GABAergic medium spiny neurons (reviewed in Vonsattel and DiFiglia, 1998), it is not surprising that postsynaptic changes in GABA receptors have been reported (Fujiyama *et al*., 2002; Thompson-Vest *et al*., 2003; Cepeda *et al*., 2004; Allen *et al*., 2009). Several studies have observed an up-regulation of $GABA_A$ receptor expression in models of HD (Fujiyama *et al*., 2002; Thompson-Vest *et al*., 2003; Cepeda *et al*., 2004; Allen *et al*., 2009). It was suggested that there was an increased contribution of $GABA_A$ receptors to synaptic transmission in the R6/2 model of HD as the frequency of spontaneous GABAergic synaptic currents was increased in medium spiny neurons in the striatum of the R6/2 mouse (Cepeda *et al*., 2004). Furthermore, this change to inhibitory synaptic function was accompanied by an increased response to exogenous GABA application and an increased expression of $\alpha_1$-$GABA_A$ receptors (Cepeda *et al*., 2004). The alterations in GABAergic synaptic currents were concurrent with the appearance of the first overt behavioural phenotype in R6/2 mice (Cepeda *et al*., 2004). In mouse models of HD reduced brain levels of BDNF have been reported (Zuccato *et al*., 2001, 2005; Spires *et al*., 2004; Lynch *et al*., 2007).

Intriguingly, a study in the striatum further demonstrated that BDNF reduced the frequency of spontaneous GABAergic synaptic currents (Cepeda *et al*., 2004). Furthermore, in the hippocampus, BDNF rescued the suppressed LTP evident with the $Hdh^{Q111}$ mouse (Lynch *et al*., 2007). It is conceivable this effect of BDNF is a consequence of an alteration in GABA-ergic signaling. Clearly a whole-cell voltage-clamp study of the phasic and tonic GABA-ergic signaling in CA1 of the $Hdh^{Q111}$ mouse would be invaluable in this regard.

Studies using alternative models of HD also report a perturbation of $GABA_A$ receptors. Increased $GABA_A$ receptor expression has been noted in the substantia nigra of rats with quinolinic acid lesions (Fujiyama *et al*., 2002). These rats exhibit a similar loss of striatonigral GABAergic neurons to that which occurs in HD and, as such, have been used as models of this disease (for references see Brickell *et al*., 1999). Specifically, it was shown in this lesion model of HD that the expression of $\beta_{2/3}$-$GABA_A$ receptors was increased in individual synapses of the substania nigra (Fujiyama *et al*., 2002). A similar increase in $GABA_A$ receptor expression has also been observed in the globus pallidus of human patients with HD, specifically demonstrated by an increase in $\alpha_1$, $\beta_{2/3}$ and $\gamma_2$-$GABA_A$ receptors (Thompson-vest *et al*., 2003; Allen *et al*., 2009). GABAergic neurons in the striatum project to both the substania nigra and globus pallidus, so it is possible that the increase in expression of the $\alpha_1$-, $\beta_{2/3}$- and $\gamma_2$-$GABA_A$ receptors in these areas is to compensate for the loss of striatal GABAergic neurons.

As mentioned briefly above, clearly a comprehensive investigation (utilising whole-cell voltage-clamp and immunohistochemistry) of the expression of synaptic and extrasynaptic $GABA_A$ receptors in WT and $Hdh^{Q111}$ mice with an emphasis on

extrasynaptic $\alpha_5$-$GABA_A$ receptors is required. Importantly, previous studies have indicated that $\alpha_5$-$GABA_A$ receptors mediate the tonic current in the hippocampus (Bai *et al*., 2001; Caraiscos *et al*., 2004; Vargas-Caballero *et al*., 2010), and exist in combination with $\beta_{1/3}$- and $\gamma_2$-subunits (Herd *et al*., 2008). Therefore, in common with the substania nigra and globus pallidus, it is possible that $\beta_3$-$GABA_A$ receptor expression, in combination with the $\alpha_5$-subunit, could also be increased in the hippocampus in HD. It would therefore be invaluable to use antibodies specific for $\alpha_5$-$GABA_A$ receptors to assess expression in the CA1 region of the hippocampus of $Hdh^{Q111}$ mice.

In order to assess the role of $\alpha_5$-$GABA_A$ receptors in tonic conductance, synaptic plasticity and memory, a study by Martin *et al.* (2010) examined synaptic plasticity and hippocampus-dependent cognition in mice null for the $\alpha_5$-$GABA_A$ receptor (*Gabra* $5^{-/-}$). Results indicated that genetic deletion or pharmacological inhibition of $\alpha_5$-$GABA_A$ receptors markedly reduces the threshold for LTP, as indicated by enhanced LTP in *Gabra5*$^{-/-}$ mice. Furthermore, it was demonstrated that $\alpha_5$-$GABA_A$ receptors had greater influence on the control of LTP at stimulation frequencies associated with theta-frequency, which has been linked to hippocampus-dependent learning (Buzsaki, 2005). In agreement, *Gabra5*$^{-/-}$ mice showed increased freezing scores in the hippocampus-dependent trace fear conditioning task (Martin *et al*., 2010), consistent with findings from a previous study in a second genetic model of $\alpha_5$-$GABA_A$ receptor deficiency (Crestani *et al*., 2002). If the $Hdh^{Q111}$ mouse demonstrates increased $\alpha_5$-$GABA_A$ receptor expression within the hippocampus, this may increase the threshold for LTP induction, perhaps underlying or contributing to the LTP deficit associated with $Hdh^{Q111}$ mice. Interestingly, as described previously, it was speculated that the *huntingtin*

mutation increases the threshold for LTP induction in the 72/ 80 CAG mouse model (Usdin *et al*., 1999; see section 7.1). As decreased $\alpha_5$-$GABA_A$ receptor expression has been associated with enhanced cognitive performance, increased $\alpha_5$-$GABA_A$ receptor expression should therefore inhibit hippocampal-dependent cognitive tasks, perhaps explaining the cognitive deficit in the $Hdh^{Q111}$ mouse.

# 9: Summary and conclusions

In humans, the clinical diagnosis of HD relies on the manifestation of motor abnormalities. In an attempt to characterise the motor phenotype of the $Hdh^{Q111}$ mouse, the circular running tracks and the activity box were used to assess locomotor activity while the rotarod was used to assess motor co-ordination. In agreement with other studies that have indicated no locomotor dysfunction in the activity box (Wheeler *et al*., 2000; Menalled *et al*., 2009), I have demonstrated that the $Hdh^{Q111}$ mouse shows no impairments in spontaneous locomotor activity in the activity box and circular runways up to the age of 13 months. Analysis of the performance on the accelerating rotarod paradigm suggested that, in agreement with Mennalled and colleagues (2009), $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice demonstrated an enhanced performance at early ages when compared to WT, which could be indicative of the hyperkinesia associated with patients in early stages of HD. In addition, I demonstrated a mild impairment in $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice from 6 and 7 months respectively, although this was not consistent at all of the subsequent ages. Tests, including the fixed speed rotarod paradigm, balance beam and foot print analysis have been used to assess fine motor co-ordination and balance in other mouse models of HD (Carter *et al*., 1999; Hickey *et al*., 2005; Stack *et al*., 2005; Hickey *et al*., 2008) and could perhaps be used to further examine the early motor phenotype of the $Hdh^{Q111}$ mouse. Nevertheless, as this thesis is focusing on the cognitive and LTP deficits that have been demonstrated prior to a motor phenotype, it is not necessarily critical that an overt motor phenotype is displayed.

Studies have shown that cognitive deficits are often present in advance of motor symptoms in human patients (Hahn-Barma *et al.*, 1998; Lawrence *et al.*, 1998;

Kirkwood *et al*., 2000; Verny *et al*., 2007) and in mouse models (Lione *et al*., 1999; Van Raamsdonk *et al*., 2005; Brooks *et al*., 2006; Pang e*t al*., 2006; Nithianantharajah *et al*., 2008; Simmons *et al*., 2009) of HD. Deficits in hippocampal LTP have also been reported at similar ages in other mouse models of HD, including the 80CAG, R6/2 $Hdh^{Q92}$, $Hdh^{Q111}$ and $Hdh^{Q140}$ mice (Usdin *et al*., 1999; Murphy *et al*., 2000; Lynch *et al*., 2007; Simmons *et al*., 2009). In agreement, I have demonstrated that, despite possessing normal basal synaptic transmission, $Hdh^{Q111+/+}$ and $Hdh^{Q111+/-}$ mice showed impairments in LTP by 2 months of age. I have also demonstrated that the $Hdh^{Q111}$ mouse model of HD displays normal cognitive processes at the age of 1 month but presents with short-term impairments in episodic memory and long-term recognition memory by 2 months. Although this is an age where $Hdh^{Q111}$ mice appeared to show enhanced performance on the rotarod, the lack of differences in the exploration patterns in the cognition test suggests that the rotarod results are not necessarily an accurate assessment of the motor phenotype of the $Hdh^{Q111}$ mice and therefore more investigation is perhaps required.

I have shown that the short-term memory deficits present at 2 months are progressive, with cognitive deficits spreading to include the object-place and object-context tasks by 13 months. It would be beneficial to characterise the brain regions involved in each of the tasks by examining the expression of cFos, following up with lesion studies in control animals, targeting the specific brain regions associated with each task in an attempt to anatomically define the cognitive deficits seen in the $Hdh^{Q111}$ mice.

The episodic and long-term recognition deficits of the $Hdh^{Q111}$ mouse were rescued following treatment with the $\alpha_5$-$GABA_A$ receptor inverse agonist $\alpha_5$IA. It would

therefore be beneficial to assess hippocampal $\alpha_5$-$GABA_A$ receptor expression using antibodies specific for the $\alpha_5$-subunit to determine expression of extrasynaptic receptors and whole-cell voltage-clamp studies to compare the properties of the tonic current in $Hdh^{Q111}$ and WT CA1 neurons in order to further examine the mechanism behind the cognitive and LTP deficits associated with HD.

In conclusion, prior to the emergence of a conclusive motor phenotype, $Hdh^{Q111}$ mice demonstrate impairments in episodic memory and deficits in the hippocampal-dependent 24 hour novel object recognition task. These findings suggest abnormal hippocampal functions early in HD. These cognitive impairments were accompanied by deficits in hippocampal LTP. Importantly, the clinically relevant heterozygous $Hdh^{Q111}$ mice exhibited an identical phenotype to homozygous $Hdh^{Q111}$ mice indicating that, reminiscent of the human disorder, only one copy of the mutant gene is necessary to produce abnormalities associated with the disorder, further supporting the validity of the $Hdh^{Q111}$ mouse as a clinically relevant model of HD. Furthermore, the LTP and cognitive deficits of the $Hdh^{Q111}$ mouse can be rescued following treatment with the $\alpha_5$-$GABA_A$ receptor inverse agonist $\alpha_5$IA. Collectively, this thesis provides evidence that $\alpha_5$-$GABA_A$ receptor antagonists have the potential to improve cognitive function in patients with HD.

# References

Ade KK, Janssen MJ, Ortinski PI, Vicini S. (2008). Differential tonic GABA conductances in striatal medium spiny neurons. *J Neurosci.* **28**(5):1185-97.

Ainge JA, Heron-Maxwell C, Theofilas P, Wright P, de Hoz L, Wood ER. (2006). The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behav Brain Res*. **167**(1):183-95.

Ainge JC and Langston RF. (2010). *Society for Neuroscience abstract*

Allen KL, Waldvogel HJ, Glass M, Faull RL. (2009). Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. *J Chem Neuroanat*. **37**(4):266-81

Alvarez P & Squire LR. (1994). Memory consolidation and the medial temporal lobe: A simple network model. *Pro. Natl. Acad. Sci. USA.* **91**, 7041-7045

Amaral DG, Dolorfo C, Alvarez-Royo P. (1991). Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat. *Hippocampus.* **1**(4):415-35.

Amaral D, Lavenex P. (2007). 'Hippocampal Neuroanatomy' in Andersen, P. *et al.* (ed.) The Hippocampus Book. New York : Oxford University Press

Amaral DG, Witter MP. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience.* **31**(3):571-91. Review.

Anderson P, Bliss TV, Skrede KK. (1971) Lamellar organization of hippocampal pathways. *Exp Brain Res*. **13**(2):222-38.

Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR. (2006). L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABAA receptors. *Neuropharmacology*. **51**(6):1023-9

Auerbach W, Hurlbert MS, Hilditch-Maguire P, Wadghiri YZ, Wheeler VC, Cohen SI, Joyner AL, MacDonald ME, Turnbull DH. (2001) The HD mutation causes progressive lethal neurological disease in mice expressing reduced levels of huntingtin. *Hum Mol Genet.* **10**:2515-2523.

Baddeley, A. and G. Hitch (1974). Working memory, Chapter 8, pp. 47–89. New York: Academic Press.

Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA. (2001). Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. *Mol Pharmacol*. **59**(4):814-24.

Baumann SW, Baur R, Sigel E. (2001). Subunit arrangement of gamma-aminobutyric acid type A receptors. *J Biol Chem.* **276**(39):36275-80.

Best TK, Siarey RJ, Galdzicki Z. (2007). Ts65Dn, a mouse model of Down syndrome, exhibits increased GABAB-induced potassium current. *J Neurophysiol*. **97**(1):892-900.

Bi G, Poo M. (2001). Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu Rev Neurosci*. **24**:139-66.

Bird LR, Roberts WA, Abroms B, Kit KA, Crupi C. (2003). Spatial memory for food hidden by rats (Rattus norvegicus) on the radial maze: studies of memory for where, what, and when. *J Comp Psychol.* **117**(2):176-87.

Bliss T, Lømo T. (1973). "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path". *J Physiol* **232**(2): 331–56.

Bliss TVP & Collingridge GL. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. **361**: 31-39.

Bliss TVP, Collingridge G & Morris R. (2007). 'Synaptic plasticity in the hippocampus' in Andersen, P. *et al* (ed.) The Hippocampus Book. New York: Oxford University Press.

Borghese CM, Stórustovu S, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford KA, Harris RA. (2006). The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. *J Pharmacol Exp Ther*. **316**(3):1360-8.

Bramham CR, Messaoudi E. (2005). BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol*. **76**(2):99-125.

Braudeau J, Delatour B, Duchon A, Pereira PL, Dauphinot L, de Chaumont F, Olivo-Marin JC, Dodd RH, Hérault Y, Potier MC. (2011). Specific targeting of the GABA-A receptor α5 subtype by a selective inverse agonist restores cognitive deficits in Down syndrome mice. *J Psychopharmacol*. **25**(8):1030-42.

Brickell KL, Nicholson LF, Waldvogel HJ, Faull RL. (1999). Chemical and anatomical changes in the striatum and substantia nigra following quinolinic acid lesions in the striatum of the rat: a detailed time course of the cellular and GABA(A) receptor changes. *J Chem Neuroanat*. **7(**2):75-97.

Broadbent NJ, Squire LR, Clark RE. (2004). Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci USA*. **101**(40):14515-20

Brooks SP, Betteridge H, Trueman RC, Jones L, Dunnett SB. (2006). Selective extra-dimensional set shifting deficit in a knock-in mouse model of Huntington's disease. *Brain Res Bull*. **69**(4):452-7.

Brown MW and Aggleton JP. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat. Rev. Neurosci.*, **2**:51–61

Brünig I, Scotti E, Sidler C, Fritschy JM. (2002). Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. *J Comp Neurol*. **443**(1):43-55.

Bussey TJ, Duck J, Muir J, Aggleton P. (2000). Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal cortices in the rats. *Behavioural Brain Research* **111**:187-202.

Butters N, Albert MS, Sax DS, Miliotis P, Nagode J & Sterste A. (1983). The effect of verbal mediators on the pictorial memory of brain-damaged patients. *Neuropsychologia*, **21**(4):307–323.

Butters N, & Grady M. (1977). Effect of predistractor delays on the shortterm memory performance of patients with Korsakoff's and Huntington's disease. *Neuropsychologia*, **15**(4/5):701–706.

Butters N, Sax D, Montgomery K & Tarlow S. (1978). Comparison of the neuropsychological deficits associated with early and advanced Huntington's disease. *Archives of Neurology*, **35**(9):585–589.

Butters N, Wolfe J, Martone M, Granholm E & Cermak LS. (1985). Memory disorders associated with Huntington's disease: Verbal recall, verbal recognition and procedural memory. *Neuropsychologia*, **23**(6), 729–743.

Caine ED, Hunt RD, Weingartner H & Ebert MH. (1978). Huntington's dementia. Clinical and neuropsychological features. *Archives of General Psychiatry*, **35**(3):377–384.

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA. (2004). Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA.* **101**(10):3662-7.

Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999). Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *J Neurosci* **19**:3248–3257.

Cattaneo E, Zuccato C, Tartari M. (2005). Normal huntingtin function: an alternative approach to Huntington's disease. *Nat Rev Neurosci* **6**:919-930.

Cepeda C, Starling AJ, Wu N, Nguyen OK, Uzgil B, Soda T, André VM, Ariano MA, Levine MS. (2004). Increased GABAergic function in mouse models of Huntington's disease: reversal by BDNF. *J Neurosci Res*. **78**(6):855-67.

Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, Reeve AJ, MacLeod AM. (2003). Identification of a novel, selective $GABA_A$ alpha5 receptor inverse agonist which enhances cognition. *J Med Chem*. **46**(11):2227-40.

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci.* **20**:8853– 8860.

Clayton NS, Dickinson A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature.* **395**(6699):272-4.

Coesman M, Weber JT, De Zeeuw CI & Hansel C. (2004). Bidirectional parallel fibre plasticity in the cerebellum under climbing fibre control. *Neuron.* **44** : 691 – 700.

Collingridge GL, Kehl SJ, McLennan H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus**.** *J Physiol.* **334**:33-46.

Collinson N, Atack JR, Laughton P, Dawson GR, Stephens DN. (2006). An inverse agonist selective for alpha5 subunit-containing GABAA receptors improves encoding and recall but not consolidation in the Morris water maze. *Psychopharmacology (Berl).* **188**(4):619-28.

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW. (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. *J Neurosci.* **22**(13):5572-80.

Cooke SF, Bliss TV. (2006). Plasticity in the human central nervous system. *Brain.* **129**(Pt 7):1659-73.

Corkin S. (2002). What's new with the amnesic patient H.M.? *Nat Rev Neurosci*. **3**(2):153-60.

Corkin S, Amaral DG, Gonzalez RG, Johnson KA and Hyman BT. (2002). H.M.'s medical temporal lobe lesion: Findings from Magnetic Resonance Imaging. *J Neurosci*.**17**(10):3964-3979.

Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Blüthmann H, Möhler H, Rudolph U. (2002). Trace fear conditioning involves hippocampal alpha5 $GABA_A$ receptors. *Proc Natl Acad Sci USA.* **25**;99(13):8980-5.

Cromer BA, Morton CJ, Parker MW. (2002). Anxiety over GABA(A) receptor structure relieved by AChBP. *Trends Biochem Sci.* **27**(6):280-7.

Cummings DM, André VM, Uzgil BO, Gee SM, Fisher YE, Cepeda C, Levine MS. (2009). Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. *J Neurosci.* **29**(33):10371-86.

Cummings DM, Milnerwood AJ, Dallérac GM, Vatsavayai SC, Hirst MC, Murphy KP. (2007). Abnormal cortical synaptic plasticity in a mouse model of Huntington's disease. *Brain Res Bull.* **72**(2-3):103-7.

Davies CH, Collingridge GL. (1996). Regulation of EPSPs by the synaptic activation of GABAB autoreceptors in rat hippocampus. *J Physiol.* **496** (2), 451-70.

Davies SN, Collingridge GL. (1989). Role of excitatory amino acid receptors in synaptic transmission in area CA1 of rat hippocampus. *Proc R Soc Lond B Biol Sci*. **236**(1285):373-84.

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell*. **90**:537–548.

Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ,Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR. (2006). An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. *J Pharmacol Exp Ther*. **316**(3), 1335-45.

Day HE, Kryskow EM, Nyhuis TJ, Herlihy L, Campeau S. (2008). Conditioned fear inhibits c-fos mRNA expression in the central extended amygdala. *Brain Res.* **1229**:137-46.

Debanne D, Guerineau NC, Giihwiler BH, Thompson SM. (1996). Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *Journal of Physiology* **491**(1):163-176.

Delis DC, Massman PJ, , Cermak LS & Kramer JH. (1991). Profiles of demented and amnesic patients on the California Verbal Learning Test: Implications for the assessment of memory disorders. *Psychological Assessment* **3**, 19–23.

Dere E, Huston JP, De Souza Silva MA. (2005). Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain Res Brain Res Protoc*. **16**(1-3):10-9.

Derry JM, Dunn SM, Davies M. (2004). Identification of a residue in the gamma-aminobutyric acid type A receptor alpha subunit that differentially affects diazepam-sensitive and -insensitive benzodiazepine site binding. *J Neurochem.* **88**(6):1431-8.

Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, Goodacre S, Lincoln RJ, Cook SM, Conley R, Hallett D, Humphries AC, Thompson SA, Wafford KA, Street LJ, Castro JL, Whiting PJ, Rosahl TW, Atack JR, McKernan RM, Dawson GR, Reynolds DS. (2005). Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines. *J Neurosci*. **25**(46):10682-8.

DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, Martin E, Vonsattel JP, Carraway R, Reeves SA, et al. (1995). Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain. *Neuron.* **14**(5):1075-81.

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and distrophic neuritis in brain. *Science.* **277**(5334); 1990-3.

DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, Martin E, Vonsattel JP, Carraway R, Reeves SA. (1999). Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron*. **14**(5):1075-81.

Dix SL and Aggleton JP. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural brain research.* **99**:191-200.

Dixon CI, Morris HV, Breen G, Desrivieres S, Jugurnauth S, Steiner RC, Vallada H, Guindalini C, Laranjeira R, Messas G, Rosahl TW, Atack JR, Peden DR, Belelli D, Lambert JJ, King SL, Schumann G, Stephens DN. (2010). Cocaine effects on mouse incentive-learning and human addiction are linked to alpha2 subunit-containing GABAA receptors. *Proc Natl Acad Sci U S A.* **107**(5):2289-94.

Dorow R, Horowski R, Paschelke G, Amin M. (1983). Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors. *Lancet.* **2**(8341):98-9.

Dragunow M, Faull R. (1989). The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods.* **29**(3), 261-5.

Du AT, Schuff N, Amend D, Laakso MP, Hsu YY, Jagust WJ, Yaffe K, Kramer JH, Reed B, Norman D, Chui HC, Weiner MW. (2001). Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J Neurol Neurosurg Psychiatry.* **71**(4):441-7.

Dunham NW, Miya TS. (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharm Assoc Am Pharm Assoc (Baltim).* **46**(3):208-9.

Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel JP, Gusella JF, Joyner AL. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science.* **269**, 407-410

Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, Folstein S, Ross C, Franz M, Abbott M, et al. (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat Genet.* **4**(4):387-92.

Eacott MJ & Norman G. (2004). Integrated memory for object, place, and context in rats: A possible model of episodic-like memory? *J Neurosci.* **24**(8), 1948-1953.

Ennaceur A & Delacour J. (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioural data. *Behavioural Brain Research.* **31**, 47-59.

Ennaceur A, Neave N, Aggleton JP. (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behavioural Brain Research.* **80**:9-25.

Ennaceur A, Neave N, Aggleton JP. (1997). Spontaneous object recognition and object location memory in rats. The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp Brain Res* **113**, 509-519.

Eustache F, Piolino P, Giffard B, Viader F, de La Sayette V, Baron JC, Desgranges B. (2004). 'In the course of time': a PET study of the cerebral substrates of autobiographical amnesia in Alzheimer's disease. *Brain.* **127**:1549–1560.

Fan MM, Fernandes HB, Zhang LY, Hayden MR, Raymond LA. (2007). Altered NMDA receptor trafficking in a yeast artificial chromosome transgenic mouse model of Huntington's disease. *J. Neurosci.* **27**:3768–3779.

Fan MM. & Raymond LA. (2007). N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog. Neurobiol.* **81**:272-293.

Farrar SJ, Whiting PJ, Bonnert TP, McKernan RM. (1999). Stoichiometry of a ligand-gated ion channel determined by fluorescence energy transfer. *J Biol Chem.* **274**(15):10100-4.

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC. (2007). Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat Neurosci.* **10**(4):411-3.

Ferrante RJ, Kowall NW, Richardson EP Jr. (1991). Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry. *J Neurosci.* **11**(12):3877-87.

Fritschy JM, Johnson DK, Mohler H, Rudolph U. (1998). Independent assembly and subcellular targeting of GABA(A)-receptor subtypes demonstrated in mouse hippocampal and olfactory neurons in vivo. *Neurosci Lett.* **249**(2-3):99-102.

Fujiyama F, Stephenson FA, Bolam JP. (2002). Synaptic localization of GABA(A) receptor subunits in the substantia nigra of the rat: effects of quinolinic acid lesions of the striatum. *Eur J Neurosci.* **15**(12):1961-75.

Gaffan, D. (1991). Spatial organization of episodic memory. *Hippocampus* **1**(3):262–264.

Gaffan, D. (1994). Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. *Journal of Cognitive Neuroscience* **6**, 305–320.

Gaffan, D. and S. Harrison (1989). Place memory and scene memory: effects of fornix transection in the monkey. *Exp Brain Res* **74**(1), 202–212.

Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, De Mey J, MacDonald ME, Lessmann V, Humbert S, Saudou F. (2004). Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell.* **118**(1):127-38.

Gines S, Seong IS, Fossale E, Ivanova E, Trettel F, Gusella JF, Wheeler VC, Persichetti F, MacDonald ME. (2003). Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. *Hum Mol Genet.* **12**(5):497-508.

Gómez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci.* **16**(14):4491-500.

Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, Bromm M, Kazemi-Esfarjani P, Thornberry NA, Vaillancourt JP, Hayden MR. (1996). Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat Genet.* **13**(4):442-9.

Graveland GA, Williams RS, DiFiglia M. (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science*. **227**(4688):770-3.

Gray M, Shirasaki DI, Cepeda C, André VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW. (2008). Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. *J Neurosci.* **28**(24):6182-95.

Guillaud L, Setou M, and Hirokawa N. (2003). KIF17 Dynamics and Regulation of NR2B Trafficking in Hippocampal Neurons. *The Journal of Neuroscience*. **23**(1):131–140

Guidetti P, Charles V, Chen EY, Reddy PH, Kordower JH, Whetsell WO Jr, Schwarcz R, Tagle DA. (2001). Early degenerative changes in transgenic mice expressing mutant huntingtin involve dendritic abnormalities but no impairment of mitochondrial energy production. *Exp Neurol.* **169**(2):340-50.

Gutekunst CA, Li SH, Yi H, Ferrante RJ, Li XJ, Hersch SM. (1998). The cellular and subcellular localization of huntingtin-associated protein 1 (HAP1): comparison with huntingtin in rat and human. *J Neurosci.* **18**(19):7674-86.

Gutekunst CA, Levey AI, Heilman CJ, Whaley WL, Yi H, Nash NR, Rees HD, Madden JJ, Hersch SM. (1995). Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. *Proc Natl Acad Sci USA.* **92**(19):8710-4.

Hahn-Barma V, Deweer B, Dürr A, Dodé C, Feingold J, Pillon B, Agid Y, Brice A, Dubois B. (1998). Are cognitive changes the first symptoms of Huntington's disease? A study of gene carriers. *J Neurol Neurosurg Psychiatry.* **64**(2):172-7.

Hamilton JM, Salmon DP, Corey-Bloom J, Gamst A, Paulsen JS, Jerkins S, Jacobson MW, Peavy G. (2003). Behavioural abnormalities contribute to functional decline in Huntington's disease. *J Neurol Neurosurg Psychiatry.* **74**(1):120-2.

Heng MY, Tallaksen-Greene SJ, Detloff PJ, Albin RL. (2007). Longitudinal evaluation of the Hdh(CAG)150 knock-in murine model of Huntington's disease. *J Neurosci.* **27**(34):8989-98.

Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D. (2008). The expression of GABAA beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. *J Physiol.* **586**(4):989-1004.

Heynen AJ, Quinlan EM, Bae DC, Bear MF. (2000). Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. *Neuron.* **28**(2):527-36.

Hickey MA, Gallant K, Gross GG, Levine MS, Chesselet MF. (2005). Early behavioral deficits in R6/2 mice suitable for use in preclinical drug testing. *Neurobiol Dis.* **20**(1):1-11.

Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, Chesselet MF (2008). Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. *Neuroscience.* **157**(1):280-95.

Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, Smith DJ, Bissada N, McCutcheon K, Nasir J, Jamot L, Li XJ, Stevens ME, Rosemond E, Roder JC, Phillips AG, Rubin EM, Hersch SM, Hayden MR. (1999). A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron.* **23**(1):181-92.

Houser CR, Esclapez M. (2003). Downregulation of the alpha5 subunit of the GABA(A) receptor in the pilocarpine model of temporal lobe epilepsy. *Hippocampus.* **13**(5):633-45.

Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR. (1998). Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci.* **1**(7):595-601.

Jacob TC., Moss SJ & Jurd R. (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* 9, 331-343.

Jones BJ, Roberts DJ. (1968). The quantiative measurement of motor inco-ordination in naive mice using an acelerating rotarod. *J Pharm Pharmacol*. **20**(4):302-4.

Kandel ER. (2009). The biology of memory: a forty-year perspective. *J Neurosci.* **29**(41):12748-56.

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P. (2010). Quantitative localisation of synaptic and extrasynaptic GABAA receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. *Eur J Neurosci.* **32**(11):1868-88.

Killoran A, Biglan KM. (2012). Therapeutics in Huntington's Disease. *Curr Treat Options Neurol.* [Epub ahead of print]

Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC. (2004). Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. *J Neurosci.* **24**(37):8153-60.

Kirkwood SC, Siemers E, Bond C, Conneally PM, Christian JC, Foroud T. (2000). Confirmation of subtle motor changes among presymptomatic carriers of the Huntington disease gene. *Arch Neurol.* **57**(7):1040-4.

Kirkwood SC, Siemers E, Hodes ME, Conneally PM, Christian JC, Foroud T. (2000). Subtle changes among presymptomatic carriers of the Huntington's disease gene. *J Neurol Neurosurg Psychiatry.* **69**(6):773-9.

Kittler JT, Thomas P, Tretter V, Bogdanov YD, Haucke V, Smart TG, Moss SJ. (2004). Huntingtin-associated protein 1 regulates inhibitory synaptic transmission by modulating gamma-aminobutyric acid type A receptor membrane trafficking. *Proc Natl Acad Sci USA.* **101**(34):12736-41.

Korpi ER, Gründer G, Lüddens H. (2002). Drug interactions at GABA(A) receptors. *Prog Neurobiol.* **67**(2):113-59.

Kramár EA, Lin B, Lin CY, Arai AC, Gall CM, Lynch G. (2004). A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J Neurosci.* **24**(22):5151-61.

Kramár EA, Lin B, Rex CS, Gall CM, Lynch G. (2006). Integrin-driven actin polymerization consolidates long-term potentiation. *Proc Natl Acad Sci USA.* **103**(14):5579-84.

Langston RF & Wood ER. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus* **20**:1139-1153 .

Langston RF, Stevenson CH, Wilson CL, Saunders I, Wood ER. (2010).The role of hippocampal subregions in memory for stimulus associations. *Behav Brain Res.* **215**(2):275-91.

Lauterborn JC, Lynch G, Vanderklish P, Arai A, Gall CM. (2000). Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J Neurosci.* **20**(1):8-21.

Lawrence AD, Hodges JR, Rosser AE, Kershaw A, ffrench-Constant C, Rubinsztein DC, Robbins TW, Sahakian BJ. (1998). Evidence for specific cognitive deficits in preclinical Huntington's disease. *Brain.* **121**(Pt 7):1329-41.

Li XJ. & Li SH. (2005). HAP1 and intracellular trafficking. *Trends Pharmacol. Sci.* **26**:1-3.

Li XJ, Li SH, Sharp AH, Nucifora FC Jr, Schilling G, Lanahan A, Worley P, Snyder SH, Ross CA. (1995). A huntingtin-associated protein enriched in brain with implications for pathology. *Nature*. **378**(6555):398-402.

Lin B, Kramár EA, Bi X, Brucher FA, Gall CM, Lynch G. (2005). Theta stimulation polymerizes actin in dendritic spines of hippocampus. *J Neurosci.* **25**(8):2062-9.

Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ. (2001). Neurological abnormalities in a knock-in mouse model of Huntington's disease. *Hum Mol Genet* **10**:137-144.

Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB. (1999). Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. *J Neurosci.* **19**(23):10428-37.

Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rülicke T, Bluethmann H, Möhler H, Rudolph U. (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science.* **290**(5489):131-4

Lüesse HG, Schiefer J, Spruenken A, Puls C, Block F, Kosinski CM. (2001). Evaluation of R6/2 HD transgenic mice for therapeutic studies in Huntington's disease: behavioral testing and impact of diabetes mellitus. *Behav Brain Res.* **126**(1-2):185-95.

Lundervold AJ, Reinvang, I, & Lundervold A. (1994). Characteristic patterns of verbal memory function in patients with Huntington's disease. Scandinavian *Journal of Psychology*, **35**(1):38–47.

Lynch G, Kramar EA, Rex CS, Jia Y, Chappas D, Gall CM, Simmons DA. (2007). Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. *J Neurosci.* **27**(16):4424-34.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F. 1983. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature.* **305** (5936):719-21.

McCormack T, Brown GD, Maylor EA, Darby RJ, Green D. (1999). Developmental changes in time estimation: comparing childhood and old age. *Dev Psychol.* **35**(4):1143-55.

MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, Barnes G, Taylor SA, James M, Groot N, MacFarlane H, Jenkins B, Anderson MA, Wexler NS and Gusella JF. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell.* **72**(6):971-83.

MacDonald RL, Olsen RW. (1994). GABAA receptor channels. *Annu Rev Neurosci.* **17**:569-602.

MacDonald RL, Rogers CJ, Twyman RE. (1989). Barbiturate regulation of kinetic properties of the GABAA receptor channel of mouse spinal neurones in culture. *J Physiol.* **417**:483-500.

McHugh TJ, Blum KI, Tsien JZ, Tonegawa S, Wilson MA. (1996). Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell.* **87**(7):1339-49.

Mackintosh, N. J. (1983). Conditioning and associative learning. Oxford: Clarendon Press.

McKay PF, Foster KL, Mason D, Cummings R, Garcia M, Williams LS, Grey C, McCane S, He X, Cook JM, June HL. (2004). A high affinity ligand for GABAA-receptor containing alpha5 subunit antagonizes ethanol's neurobehavioral effects in Long-Evans rats. *Psychopharmacology (Berl).* **172**(4):455-62.

McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L,Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor alpha1 subtype. *Nat Neurosci.* **(6):**587-92.

McNamara RK, dePape GE, Skelton RW. (1993). Differential effects of benzodiazepine receptor agonists on hippocampal long-term potentiation and spatial learning in the Morris water maze. *Brain Res.* **626**(1-2):63-70.

McNamara RK, Skelton RW. (2002). Benzodiazepine receptor antagonists flumazenil and CGS 8216 and inverse agonist β-CCM enhance spatial learning in the rat: dissociation from anxiolytic actions. *Psychobiology*. **21**(2). 101-108.

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell.* **87**(3):493-506.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. (2004). Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci.* **5**:793–807.

Martin EJ, Kim M, Velier J, Sapp E, Lee HS, Laforet G, Won L, Chase K, Bhide PG, Heller A, Aronin N, Difiglia M. (1999). Analysis of Huntingtin-associated protein 1 in mouse brain and immortalized striatal neurons. *J Comp Neurol*. **403**(4):421-30.

Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA. (2010). Alpha5 $GABA_A$ receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. *J Neurosci.* **30**(15):5269-82.

Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, McCutcheon K, Singaraja R, Kazemi-Esfarjani P, Devon R, Kim SU, Bredesen DE, Tufaro F,Hayden MR. (1998). Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat Genet.* **18**(2):150-4.

Mega MS. (2001). The entorhinal cortex in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. **71**(4):431-2

Melone MA, Jori FP, Peluso G. (2005). Huntington's disease: new frontiers for molecular and cell therapy. *Curr Drug Targets*. **6**(1):43-56.

Menalled LB, Sison JD, Wu Y, Olivieri M, Li XJ, Li H, Zeitlin S, Chesselet MF (2002) Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. *J Neurosci.* **22**: 8266–8276.

Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. (2003). Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. *J Comp Neurol.* **465**: 11–26

Menalled L, El-Khodor BF, Patry M, Suárez-Fariñas M, Orenstein SJ, Zahasky B, Leahy C, Wheeler V, Yang XW, MacDonald M, Morton AJ, Bates G, Leeds J,Park L, Howland D, Signer E, Tobin A, Brunner D. (2009). Systematic behavioral evaluation of Huntington's disease transgenic and knock-in mouse models. *Neurobiol Dis.* **35**(3): 319-36

Meunier M, Bachevalier J, Mishkin M, and Murray EA. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J Neurosci* **13**(12):5418–5432.

Milnerwood AJ, Cummings DM, Dallerac GM, Brown JY, Vatsavayai SC, Hirst MC, Rezaie P, Murphy KP. (2006). Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. *Hum Mol Genet* **15**:1690–1703.

Milnerwood AJ, Gladding CM, Pouladi MA, Kaufman AM, Hines RM, Boyd JD, Ko RW, Vasuta OC, Graham RK, Hayden MR, Murphy TH, Raymond LA. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron.* **65**(2):178-90.

Mishkin M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature.* **273**(5660):297-8.

Möhler H. (2007). Molecular regulation of cognitive functions and developmental plasticity: impact of GABAA receptors. *J Neurochem.* **102**(1):1-12

Montoya A, Pelletier M, Menear M, Duplessis E, Richer F, Lepage M. (2006). Episodic memory impairment in Huntington's disease: a meta-analysis. *Neuropsychologia.* **44**(10):1984-94.

Montoya A, Price BH, Menear M, Lepage M. (2006). Brain imaging and cognitive dysfunctions in Huntington's disease. *J Psychiatry Neurosci.* **31**(1):21-9.

Monville C, Torres EM, Dunnett SB. (2006). Comparison of incremental and accelerating protocols of the rotarod test for the assessment of motor deficits in the 6-OHDA model. J Neurosci Methods. **158**(2):219-23.

Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature.* **354**(6348):31-7

Morris RG, Anderson E, Lynch GS, Baudry M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature.* **319**(6056):774-6.

Morton AJ, Hunt MJ, Hodges AK, Lewis PD, Redfern AJ, Dunnett SB, Jones L. (2005). A combination drug therapy improves cognition and reverses gene expression changes in a mouse model of Huntington's disease. *Eur J Neurosci.* **21**(4):855-70.

Morton AJ, Lagan MA, Skepper JN, Dunnett SB. (2000). Progressive formation of inclusions in the striatum and hippocampus of mice transgenic for the human Huntington's disease mutation. *J Neurocytol.* **29**(9):679-702.

Moscovitch M. Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G, and Nadel L. (2005). Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *J Anat.* **207**(1), 35–66.

Moss MB, Albert MS, Butters N & Payne M. (1986). Differential patterns of memory loss among patients with Alzheimer's disease, Huntington's disease, and alcoholic Korsakoff's syndrome. *Archives of Neurology*, **43**(3):239–246.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE and Lehmann H. (2002). Hippocampal damage and exploratory preference in rats: Memory for objects, places and contexts. *Learning and memory*. **9**:49-57

Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (2000) Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. *J Neurosci.* **20**:5115–5123.

Murray EA and Mishkin M. (1986). Visual recognition in monkeys following rhinal cortical ablations combined with either amygdalectomy or hippocampectomy. *J Neurosci*. **6**(7):1991–2003.

Nadel L, Moscovitch M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol*. **7**(2):217-27. Review.

Nance MA, Myers RH. (2001). Juvenile onset Huntington's disease-clinical and research perspectives. *Ment Retard Dev Disabil Res Rev*. **7**(3):153-7. Review

Nasir J, Floresco SB, O'Kusky JR, Diewert VM, Richman JM, Zeisler J, Borowski A, Marth JD, Phillips AG, Hayden MR. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell*. **81**(5):811-23

Naver B, Stub C, Møller M, Fenger K, Hansen AK, Hasholt L, Sørensen SA. (2003). Molecular and behavioral analysis of the R6/1 Huntington's disease transgenic mouse. *Neuroscience*. **122**(4):1049-57

Nithianantharajah J, Barkus C, Murphy M, Hannan AJ. (2008). Gene-environment interactions modulating cognitive function and molecular correlates of synaptic plasticity in Huntington's disease transgenic mice. *Neurobiol Dis*. **29**(3):490-504.

Norman G & Eacott M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience.* **119**(2):557–566.

Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P. (1996). Differential synaptic localization of two major gamma-aminobutyric acid type A receptor alpha subunits on hippocampal pyramidal cells. *Proc Natl Acad Sci USA.* **93**(21):11939–11944.

Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes AR. (2007). Blockade of alcohol's amnestic activity in humans by an alpha5 subtype benzodiazepine receptor inverse agonist. *Neuropharmacology.* **53**(7):810-20.

Nyíri G, Freund TF, Somogyi P. (2001). Input-dependent synaptic targeting of alpha(2)-subunit-containing GABA(A) receptors in synapses of hippocampal pyramidal cells of the rat. *Eur J Neurosci.* **13**(3):428-42.

Okamoto S, Pouladi MA, Talantova M, Yao D, Xia P, Ehrnhoefer DE, Zaidi R, Clemente A, Kaul M, Graham RK, Zhang D, Vincent Chen HS, Tong G, Hayden MR,Lipton SA. (2009). Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat Med.* **15**(12):1407-13.

Olsen RW, Hanchar HJ, Meera P, Wallner M. (2007). GABAA receptor subtypes: the "one glass of wine" receptors. *Alcohol.* **41**(3):201-9.

Olsen RW, Sieghart W. (2008). International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev.* **60**:243–260

Olton, D. and R. Samuelson. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes* **2**, 97–116.

Otto T, Eichenbaum H, Wiener SI, Wible CG. (1991). Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation. *Hippocampus.* **1**(2):181-92.

Otto T and Eichenbaum H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed nonmatching-to-sample task. *Behav Neurosci* **106**(5):762–775.

Orvoen S, Pla P, Gardier AM, Saudou F, David DJ. (2012). Huntington's disease knock-in male mice show specific anxiety-like behaviour and altered neuronal maturation. *Neurosci Lett.* **507**(2):127-32.

Pang TY, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ. (2006). Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in Huntington's disease transgenic mice. *Neuroscience.* **141**(2):569-84

Pang TY, Du X, Zajac MS, Howard ML, Hannan AJ. (2009). Altered serotonin receptor expression is associated with depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. *Hum Mol Genet.* **18**(4):753-66.

Papadia S, Hardingham GE. (2007). The dichotomy of NMDA receptor signaling. *Neuroscientist.* **13**(6):572-9.

Paulsen JS, Langbehn DR, Stout JC, Aylward E, Ross CA, Nance M, Guttman M, Johnson S, MacDonald M, Beglinger LJ, Duff K, Kayson E,Biglan K, Shoulson I, Oakes D, Hayden M; Predict-HD Investigators and Coordinators of the Huntington Study Group. (2008). Detection of Huntington's disease decades before diagnosis: the Predict-HD study. *J Neurol Neurosurg Psychiatry.* **79** (8): 874-80.

Paulsen JS. (2011). Cognitive impairment in Huntington disease: diagnosis and treatment. *Curr Neurol Neurosci Rep.* **11**(5):474-83

Pellow S, File SE. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav.* **24**(3):525-9.

Petersen EN. DMCM (1983). A potent convulsive benzodiazepine receptor ligand. *Eur J Pharmacol.* **94**:117-124.

Pickering C, Avesson L, Lindblom J, Liljequist S, Schiöth HB. (2007). Identification of neurotransmitter receptor genes involved in alcohol self-administration in the rat prefrontal cortex, hippocampus and amygdala. *Prog Neuropsychopharmacol Biol Psychiatry.* **31**(1):53-64.

Porcello DM, Huntsman MM, Mihalek RM, Homanics GE, Huguenard JR. (2003). Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking delta subunit. *J Neurophysiol.* **89**(3):1378-86.

Pouladi MA, Graham RK, Karasinska JM, Xie Y, Santos RD, Petersén A, Hayden MR. (2009). Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. *Brain.* **132**(Pt 4):919-32

Prut L, Prenosil G, Willadt S, Vogt K, Fritschy JM, Crestani F. (2010). A reduction in hippocampal GABAA receptor alpha5 subunits disrupts the memory for location of objects in mice. *Genes Brain Behav.* **9**(5):478-88.

Rex CS, Lin CY, Kramár EA, Chen LY, Gall CM, Lynch G. (2007). Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. *J Neurosci.* **27**(11):3017-29.

Rioult-Pedotti MS, Friedman D & Donoghue JP. (2000). Learning-induced LTP in neocortex. *Science.* **290**:533-536.

Romero E, Cha GH, Verstreken P, Ly CV, Hughes RE, Bellen HJ, Botas J. (2008). Suppression of neurodegeneration and increased neurotransmission caused by expanded full-length huntingtin accumulating in the cytoplasm. *Neuron.* **57**(1):27-40.

Roos RA. (2010). Huntington's disease: a clinical review. *Orphanet J Rare Dis.* **5**(1):40.

Rosas HD, Koroshetz WJ, Chen YI, Skeuse C, Vangel M, Cudkowicz ME, Caplan K, Marek K, Seidman LJ, Makris N, Jenkins BG, Goldstein JM. (2003). Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. *Neurology.* **60**(10):1615-20.

Rudolph U, Crestani F, Benke D, Brunig I, Benson J, Fritschy JM, Martin JR, Bluethmann H, Mohler H. (1999). Benzodiazepine actions mediated by specific γ-aminobutyric acid$_A$ receptor subtypes. *Nature* **401**:796-800.

Rudolph U, Möhler H. (2006). GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol.* **6**(1):18-23.

Rueda N, Flórez J, Martínez-Cué C. 2008, Chronic pentylenetetrazole but not donepezil treatment rescues spatial cognition in Ts65Dn mice, a model for Down syndrome. *Neurosci Lett.* ***433***(1):22-7.

Sarter M, Bruno JP, Berntson GG. (2001). Psychotogenic properties of benzodiazepine receptor inverse agonists. *Psychopharmacology.* **156**:1-13.

Sarto-Jackson I, Sieghart W. (2008). Assembly of GABA(A) receptors (Review). *Mol Membr Biol.* **25**(4):302-10.

Saudou F, Finkbeiner S, Devys D, Greenberg ME. (1998). Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell.* **95**(1):55-66.

Save E, Poucet B, Foreman N, Buhot MC. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behav Neurosci.* **106**(3):447-56.

Savić MM, Majumder S, Huang S, Edwankar RV, Furtmüller R, Joksimović S, Clayton T Sr, Ramerstorfer J, Milinković MM, Roth BL, Sieghart W, Cook JM. (2010). Novel positive allosteric modulators of GABAA receptors: do subtle differences in activity at alpha1 plus alpha5 versus alpha2 plus alpha3 subunits account for dissimilarities in behavioral effects in rats? *Prog Neuropsychopharmacol Biol Psychiatry.* **34**(2):376-86

Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE. (1997). Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell.* **90**(3):549-58.

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, et al. (1987). Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family. *Nature.* **328**(6127):221-7.

Schulz PE, Cook EP, Johnston D. (1994). Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. *J Neurosci.* **14**(9):5325-37.

Scoville WB & Milner B. (1957). *J Neurol. Neurosurg. Psychiat*. **20**:11-21

Seabrook GR, Easter A, Dawson GR, Bowery BJ. (1997). Modulation of long-term potentiation in CA1 region of mouse hippocampal brain slices by GABAA receptor benzodiazepine site ligands. *Neuropharmacology.* **36**(6):823-30.

Sergeeva OA, Andreeva N, Garret M, Scherer A, Haas HL. (2005). Pharmacological properties of GABAA receptors in rat hypothalamic neurons expressing the epsilon-subunit. *J Neurosci.* **25**(1):88-95.

Sharp AH, Loev SJ, Schilling G, Li SH, Li XJ, Bao J, Wagster MV, Kotzuk JA, Steiner JP, Lo A. (1995). Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron.* **14**(5):1065-74.

Shelbourne PF, Killeen N, Hevner RF, Johnston HM, Tecott L, Lewandoski M, Ennis M, Ramirez L, Li Z, Iannicola C, Littman DR, Myers RM. (1999). A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. *Hum Mol Genet.* **8**(5):763-74.

Sieghart W. (1995). Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. *Pharmacol Rev.* **47**(2):181-234.

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Höger H, Adamiker D. (1999). Structure and subunit composition of GABA(A) receptors. *Neurochem Int.* **34**(5):379-85.

Sieghart W, Sperk G. (2002). Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr Top Med Chem.* **2**(8):795-816.

Sigel E. (2002). Mapping of the benzodiazepine recognition site on GABA(A) receptors. *Curr Top Med Chem.* **2**(8):833-9.

Sigel E, Lüscher BP. (2011). A closer look at the high affinity benzodiazepine binding site on GABAA receptors. *Curr Top Med Chem.* **11**(2):241-6.

Sigel E, Stephenson FA, Mamalaki C, Barnard EA. (1983). A gamma-aminobutyric acid/benzodiazepine receptor complex of bovine cerebral cortex. *J Biol Chem.* **258**(11):6965-71

Simmons DA, Rex CS, Palmer L, Pandyarajan V, Fedulov V, Gall CM, Lynch G. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proc Natl Acad Sci USA.* **106**(12):4906-11

Skrede KK, Westgaard RH. 1971. The transverse hippocampal slice: a well-defined cortical structure maintained in vitro. *Brain Res.* **35**(2):589-93.

Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Hum Mol Genet.* **12**:1555–1567.

Solomon AC, Stout JC, Johnson SA, Langbehn DR, Aylward EH, Brandt J, Ross CA, Beglinger L, Hayden MR, Kieburtz K, Kayson E, Julian-Baros E, Duff K,Guttman M, Nance M, Oakes D, Shoulson I, Penziner E, Paulsen JS; Predict-HD investigators of the Huntington Study Group. (2007). Verbal episodic memory declines prior to diagnosis in Huntington's disease. *Neuropsychologia.* **45**(8):1767-76.

Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, Hannan AJ. (2004). Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. *J Neurosci.* **24**(9):2270-6.

Squire LR, Zola-Morgan S. (1991). The medial temporal lobe system. *Science.* **253**:1380-1386.

Squire LT. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* **99**:195-231.

Stack EC, Kubilus JK, Smith K, Cormier K, Del Signore SJ, Guelin E, Ryu H, Hersch SM, Ferrante RJ. (2005). Chronology of behavioral symptoms and neuropathological sequela in R6/2 Huntington's diseasetransgenic mice. *J Comp Neurol.* **490**(4):354-70.

Sternfeld F, Carling RW, Jelley RA, Ladduwahetty T, Merchant KJ, Moore KW, Reeve AJ, Street LJ, O'Connor D, Sohal B, Atack JR, Cook S, Seabrook G, Wafford K, Tattersall FD, Collinson N, Dawson GR, Castro JL, MacLeod AM. (2004). Selective, orally active gamma-aminobutyric acidA alpha5 receptor inverse agonists as cognition enhancers. *J Med Chem.* **47**(9):2176-9.

Sturrock A, Leavitt BR. (2010). The clinical and genetic features of Huntington disease. *J Geriatr Psychiatry Neurol.* **23**(4):243-59.

Sun Z, Del Mar N, Meade C, Goldowitz D, Reiner A. (2002). Differential changes in striatal projection neurons in R6/2 transgenic mice for Huntington's disease. *Neurobiol Dis.* **11**(3):369-85.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ. (1999). Genetic enhancement of learning and memory in mice. *Nature.* **401**(6748):63-9.

Taubenfeld SM, Wiig KA, Bear MF, Alberini CM. (1999). A molecular correlate of memory and amnesia in the hippocampus. *Nat Neurosci.* **2**(4):309-10.

Taubenfeld SM, Wiig KA, Monti B, Dolan B, Pollonini G, Alberini CM. (2001). Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein [beta] and [delta] Co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term memory consolidation. *J Neurosci.* **21**(1):84-91.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ. (1999). Genetic enhancement of learning and memory in mice. *Nature.* **401**(6748):63-9.

Thompson-Vest NM, Waldvogel HJ, Rees MI, Faull RL. (2003). GABA(A) receptor subunit and gephyrin protein changes differ in the globus pallidus in Huntington's diseased brain. *Brain Res.* **994**(2):265-70.

Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, Cattaneo E, MacDonald ME. (2000). Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. *Hum Mol Genet.* **22**;9(19):2799-809.

Trudell J. (2002). Unique assignment of inter-subunit association in GABA(A) alpha 1 beta 3 gamma 2 receptors determined by molecular modeling. *Biochim Biophys Acta.* **1565**(1):91-6.

Tsien JZ, Huerta PT, Tonegawa S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synapticplasticity in spatial memory. *Cell.* **87**(7):1327-38.

Tulving, E. (1972). Organization of memory. New York: Academic Press.

Tulving, E. (1983). Elements of Episodic Memory. Oxford: Clarendon Press.

Tulving E, Markowitsch HJ. (1998). Episodic and declarative memory: role of the hippocampus. *Hippocampus.* **8**(3):198-204.

Twelvetrees AE, Yuen EY, Arancibia-Carcamo IL, MacAskill AF, Rostaing P, Lumb MJ, Humbert S, Triller A, Saudou F, Yan Z, Kittler JT. (2010). Delivery of GABAARs to synapses is mediated by HAP1-KIF5 and disrupted by mutant huntingtin. *Neuron.* **65**(1):53-65.

Unwin N. (2005). Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol.* **346**(4):967-89.

Usdin MT, Shelbourne PF, Myers RM, Madison DV. (1999). Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum Mol Genet.* **8**(5):839-46.

VanElzakker M, Fevurly RD, Breindel T, Spencer RL. (2008). Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learn Mem.* **15**(12):899-908.

Van Raamsdonk JM, Murphy Z, Slow EJ, Leavitt BR, Hayden MR (2005a). Selective degeneration and nuclear localization of mutant huntingtin in the YAC128 mouse model of Huntington disease. *Hum Mol Genet* **14**:3823–3835

Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR. (2005b). Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. *J Neurosci.* **25**(16):4169-80.

Vargas-Caballero M, Martin LJ, Salter MW, Orser BA, Paulsen O. (2010). alpha5 Subunit-containing GABA(A) receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation. *Neuropharmacology.* **58**(3):668-75.

Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. (1997). Differential effects of early hippocampal pathology of episodic and semantic memory. *Science.* **277**:376-380.

Verny C, Allain P, Prudean A, Malinge MC, Gohier B, Scherer C, Bonneau D, Dubas F, Le Gall D. (2007). Cognitive changes in asymptomatic carriers of the Huntington disease mutation gene. *Eur J Neurol.* **14**(12):1344-50.

Vonsattel JP, DiFiglia M. (1998). Huntington disease. *J Neuropathol Exp Neurol.* **57**(5):369-84.

Wallner M, Hanchar HJ, Olsen RW. (2003). Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc Natl Acad Sci USA.* **100**(25):15218-23.

Wallner M, Hanchar HJ, Olsen RW. (2006). Low-dose alcohol actions on alpha4beta3delta GABAA receptors are reversed by the behavioral alcohol antagonist Ro15-4513. *Proc Natl Acad Sci U S A.* **103**(22):8540-5.

Wallner M, Hanchar HJ, Olsen RW. (2006). Low dose acute alcohol effects on GABA A receptor subtypes. *Pharmacol Ther.* **112**(2):513-28.

Waugh NC, Norman DA. (1965). Primary memory. *Psychol Rev.* **72**:89-104.

Wheeler VC, White JK, Gutekunst CA, Vrbanac V, Weaver M, Li XJ, Li SH, Yi H, Vonsattel JP, Gusella JF, Hersch S, Auerbach W, Joyner AL, MacDonald ME. (2000). Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. *Hum Mol Genet.* **9**(4):503-13.

Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S, Friedlander RM, Gusella JF, Vonsattel JP, Borchelt DR, MacDonald ME. (2002). Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. *Hum Mol Genet.* **15**;11(6):633-40.

White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL, MacDonald ME. (1997). Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. *Nat Genet.* **17**(4):404-10

Whitlock JR, Heynen AJ, Shuler MG, Bear MF. (2006). Learning induces long-term potentiation in the hippocampus. *Science.* **313**(5790):1093-7.

Williams JK, Barnette JJ, Reed D, Sousa VD, Schutte DL, McGonigal-Kenney M, Jarmon L, Phillips E, Tripp-Reimer T, Paulsen JS. (2010). Development of the Huntington disease family concerns and strategies survey from focus group data. *J Nurs Meas.* **18**(2):83-99.

Whishaw IQ and Tomie JA. (1991). Acquisition and retention by hippocampal rats of simple, conditional, and configural tasks using tactile and olfactory cues: implications for hippocampal function. *Behav Neurosci.* **105**(6):787–797.

Whishaw IQ, Tomie JA. (1989). Olfaction directs skilled forelimb reaching in the rat. *Behav Brain Res*. **32**(1):11-21.

Wu L & Saggau. (1994). Presynaptic calcium is increased during normal synaptic transmission and paired pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. *J Neurosci.* **14**:645-654.

Yee BK, Keist R, von Boehmer L, Studer R, Benke D, Hagenbuch N, Dong Y, Malenka RC, Fritschy JM, Bluethmann H, Feldon J,Möhler H, Rudolph U. (2005). A schizophrenia-related sensorimotor deficit links alpha 3-containing GABAA receptors to a dopamine hyperfunction. *Proc Natl Acad Sci USA.* **102**(47):17154-9.

Yu ZX, Li SH, Evans J, Pillarisetti A, Li H, Li XJ. (2003). Mutant huntingtin causes context-dependent neurodegeneration in mice with Huntington's disease. *J Neurosci.* **23**(6):2193-202.

Yuen EY, Wei J, Zhong P, Yan Z. (2012). Disrupted GABAAR trafficking and synaptic inhibition in a mouse model of Huntington's disease. *Neurobiol Dis.* **46**(2):497-502 **.**

Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, Efstratiadis A. (1995). Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat Genet.* **11**:155-163.

Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt B, Brundin P, Hayden MR, Raymond LA. (2002). Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* **33**:849–860.

Zola-Morgan S, Squire LR, Amaral DG. (1989). Lesions of the hippocampal formation but not lesion of the fornix or the mammillary nuclei produce long-lasting memory impairment in monkeys. *J Neurosci.* **9**(3):898-913.

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science.* **293**(5529):493-8.